7526

THE JOURNAL OF EXPERIMENTAL MEDICINE

THE JOURNAL OF EXPERIMENTAL MEDICINE

EDITED BY

SIMON FLEXNER, M. D.

AND

EUGENE L. OPIE, M. D.

PUBLISHED BY

THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
NEW YORK

VOLUME SEVENTH

WITH THIRTY-FIVE PLATES AND TWENTY-TWO FIGURES IN THE TEXT

NEW YORK
THE MACMILLAN COMPANY
LONDON: MACMILLAN & CO., LTD.
1905

CONTENTS

No. 1, February 25, 1905.

PAGE

I. Dawson, Percy M. The Changes in the Heart-Rate and Blood "Pressures" Resulting from Severe Hæmorrhage and Subsequent Infusion of Sodium Bicarbonate 1

II. Emerson, Haven, and Norris, Charles. "Red-Leg"—An Infectious Disease of Frogs . . . 32

III. Meltzer, S. J., and Auer, John. On the Rate of Absorption from Intramuscular Tissue 59
Plates I-III.

IV. Herter, C. A. The Color Reactions of Naphthaquinone Sodium-Monosulphonate and Some of their Biological Applications 79

V. Taylor, Alonzo Englebert. On the Preparation of Salt-Free Culture Media and the Growth of Bacteria upon them 111

No. 2, April 25, 1905.

VI. Woltmann, Harro. A Study of the Changes in the Blood and Blood-Forming Organs Produced by Cytotoxic Sera, with Special Reference to Hæmolymphotoxin 119
Plates IV and V.

VII. Sweet, J. E. The Artificial Anastomosis between the Portal Vein and the Vena Cava Inferior—Eck's Fistula 163
Plates VI-VIII.

VIII. Reichert, Edward T. A Second Coagulation of the Blood Due to a Substance that is not Identical with Fibrinogen and is Coagulable by Saturation with Neutral Oxalate 173

156932

PAGE

IX. Hubbert, William R. Comparative Statistics of Antitoxin Horses. A Study of the Records of One Hundred Horses Immunized to Diphtheria Toxin, with Composite of Curves 176

X. Farnum, Charles G. Some Observations upon Endothelial Separation in the Smaller Arteries and Veins 183

Plates IX and X.

XI. Noguchi, Hideyo. A Study of the Protective Action of Snake Venom upon Blood Corpuscles . 191

XII. Hiss, Philip Hanson, Jr. A Method for Obtaining Mass Cultures of Bacteria for Inoculation and for Agglutination Tests: with Special Reference to Pneumococci and Streptococci 223

Plate XI.

No. 3, June 10, 1905.

XIII. Strong, Richard P. Some Questions Relating to the Virulence of Micro-Organisms, with Particular Reference to their Immunizing Powers 229

Plates XII-XX.

XIV. Hastings, T. W. A Method for Preparing a Permanent Nocht's Stain (Nocht-Jenner Stain) . . 265

Plates XXI and XXII.

XV. Meltzer, S. J., and Salant, William. Studies on the Toxicity of Bile. I—The Effects of Intravenous Injections of Bile upon Blood Pressure . . 280

Plates XXIII-XXVI.

XVI. Wakeman, Alfred J. On the Hexon Bases of Liver Tissue under Normal and Certain Pathological Conditions 292

XVII. Harris, Norman MacLeod. The Construction of a Thermostat-Room 308

Plate XXVII.

XVIII. Opie, Eugene L. Enzymes and Anti-Enzymes of Inflammatory Exudates 316

No. 4, July 15, 1905.

PAGE

XIX. Wollstein, Martha. A Study of the Bacteriology of Pertussis, with Special Reference to the Agglutination of the Patient's Blood 335

XX. Field, Cyrus W. On the Presence of Certain Bodies in the Skin and Blister Fluid from Scarlet-Fever and Measles 343

Plate XXVIII.

XXI. Ricketts, H. T. Concerning the Possibility of an Antibody for the Tetanophile Receptor of Erythrocytes: A Receptor Study 351

XXII. Torrey, John C. A Comparative Study of Dysentery and Dysentery-like Organisms.

Part I.—Bacilli Producing a Typical Reaction in Litmus Milk 365

Part II.—Dysentery-like Organisms which Produce an Atypical Reaction in Litmus Milk, and which should probably be Included within the Group of Pseudo-Dysentery Bacilli . . . 385

No. 5, August 25, 1905.

XXIII. Introductory Note to Studies on the Pneumococcus under the Auspices of the Medical Commission for the Investigation of Acute Respiratory Diseases of the Department of Health of the City of New York 401

XXIV. Park, William H., and Williams, A. W. A Study of Pneumococci: A Comparison between the Pneumococci Found in the Throat Secretions of Healthy Persons Living in both City and Country, and those Obtained from Pneumonic Exudates and Diseased Mucous Membranes 403

XXV. Collins, Katherine R. The Application of the Reaction of Agglutination to the Pneumococcus . 420

XXVI. Longcope, Warfield T., and Fox, W. W. A Comparative Study of Pneumococci and Streptococci from the Mouths of Healthy Individuals and from Pathological Conditions 430

PAGE

XXVII. NORRIS, CHARLES, and PAPPENHEIMER, ALWIN M. A Study of Pneumococci and Allied Organisms in Human Mouths and Lungs after Death . . . 450

XXVIII. DUVAL, CHARLES W., and LEWIS, PAUL A. Studies on the Pneumococcus 473

Plate XXIX.

XXIX. BUERGER, LEO. Studies of the Pneumococcus and Allied Organisms with Reference to their Occurrence in the Human Mouth 497

XXX. HISS, PHILIP HANSON, Jr., assisted by BORDEN, JOHN HARVEY, and KNAPP, CLINTON BEECHAM. A Comparative Study of Pneumococci and Allied Organisms 547

XXXI. WOOD, FRANCIS CARTER. The Viability of the Pneumococcus after Drying: A Study of One of the Factors in Pneumonic Infection 592

XXXII. LONGCOPE, WARFIELD T. A Note upon the Growth of Pneumococci in Blood Serum 626

No. 6, NOVEMBER 25, 1905.

XXXIII. KLOTZ, OSKAR. Studies upon Calcareous Degeneration 633

Plates XXX and XXXI.

XXXIV. ERLANGER, JOSEPH. On the Physiology of Heart-Block in Mammals, with Especial Reference to the Causation of Stokes-Adams Disease. Part I 676

Plates XXXII-XXXV.

XXXV. KINYOUN, J. J. The Action of Glycerin on Bacteria in the Presence of Cell Exudates 725

XXXVI. BEEBE, S. P. Cytotoxic Serum Produced by the Injection of Nucleoproteids 733

XXXVII. HERRICK, FREDERICK C. A Contribution to the Technique of Making the Eck Fistula 751

XXXVIII. OPIE, EUGENE L. The Presence in the Bone-Marrow of Enzymes Resembling those of Leucocytes 759

THE CHANGES IN THE HEART RATE AND BLOOD "PRESSURES" RESULTING FROM SEVERE HÆMORRHAGE AND SUBSEQUENT INFUSION OF SODIUM BICARBONATE.

By PERCY M. DAWSON.

(*From the Physiological Laboratory of the Johns Hopkins University.*)

INTRODUCTION.

One of several conclusions reached by Howell in his study of the phenomena of shock[1] is the following: "Injections of alkaline solutions of sodium carbonate, intravenously or into the rectum during shock, increase markedly the amplitude of the heart-beat and bring about a rise of arterial pressure. When the shock is moderate (aortic tension of 60–70 mm. Hg.), the injections may restore arterial pressure to an approximately normal level. When the shock is severe (aortic tension of 20–40 mm. Hg.), the injections may increase the arterial pressure by about 100 per cent for long intervals, and the effect when it wears off may be restored by repeating the injections. The effect of the injections is due chiefly or entirely to a direct action on the heart."

Having in mind the possible clinical value of injections of alkaline solutions as a means of combating the condition of shock, Professor Howell then suggested to the author to pursue this line of investigation still farther, a suggestion which resulted in the series of experiments which will now be described.

The present communication deals with the cardio-vascular effects of intravenous infusion of solutions containing sodium bicarbonate after severe hæmorrhage. The animals used were dogs, and the phenomena observed were the pulse rate and the blood "pressures," viz., systolic, mean, diastolic, and pulse

[1] *Contributions to Medical Research*, dedicated to V. C. Vaughan, Ann Arbor, 1903, p. 61.

pressure.[2] Naturally a careful study was made of the effects, not only of the infusion, but also of the hæmorrhage which preceded it. It has therefore been thought desirable to record the phenomena accompanying the hæmorrhage even at the risk of repeating some of the statements already proved by others.

METHOD.

Anæsthesia.—Each dog received from 0.6 to 1.2 grm. of morphia hypodermically, and an hour later was anæsthetized with ether, administered at first with a cone, but later through a tracheal cannula.

Hæmorrhage.—The blood was allowed to flow through a cannula inserted into the carotid artery, until an amount equal to from 2.3 to 4.4 per cent of the body weight had been removed. The bleeding occupied as a rule about five minutes.

Infusion.—The apparatus for infusing was simple. A Marriotte's bottle containing the liquid to be infused was kept at a fairly constant temperature in a bath of warm water. This bottle was connected by means of a short rubber tube to a large glass T. Into the upright limb of this T a thermometer was inserted, while of the two ends of the cross-piece one was connected with the bottle, as already stated, and the other entered directly into the jugular vein. The rate of infusion was regulated by means of a glass stop-cock inserted in the course of the rubber tube above mentioned. The time occupied by the infusion was usually from five to six minutes, and the amount of fluid infused varied from 1.8 to 4.4 per cent of the body weight. The solutions infused were of two kinds, first that containing only sodium chloride (0.8 per cent); and secondly, that which in addition to the sodium chloride (0.8 per cent) contained different amounts of sodium bicarbonate ($\frac{1}{10}$, $\frac{1}{4}$, $\frac{1}{2}$, or 1 per cent). [3]

[2] Each ventricular contraction causes a wave of positive pressure (the pulse) to pass along the arterial tree towards the periphery. Hence the rhythmic change in arterial pressure at any given point has been designated the "pulse pressure." It is, of course, calculated by subtracting the diastolic pressure at the point in question from the corresponding systolic pressure.

[3] The criticism might be made that the effects, which later in this communication will be ascribed to the specific action of the bicarbonate, might with equal

Recording Apparatus.—As a means of securing graphic records the following scheme was adopted. The left femoral artery was connected with the Hürthle manometer, so that the pulse rate could be accurately recorded. The right femoral artery also was connected with a tube which branched into three parts, each being in communication with a separate mercury manometer. In the course of the first branch there was inserted a maximum valve; in the course of the second, a minimum valve; while the third branch ran directly to its manometer. Consequently it was possible to obtain readings of the systolic, diastolic, and mean pressures, and from the first two to calculate the pulse pressure.[4]

reason be attributed to the fact that the alkaline solutions were more or less strongly hypertonic. That this is not the true explanation of the results obtained is shown by several experiments, in some of which strongly hypertonic solutions of sodium chloride were used, while in others the solution contained chloride and bicarbonate, each being present to the extent of 5 % ("5 to 5 solution"). The former group of experiments gave the results usually obtained with isotonic chloride solution, while the effect of the latter was similar to that obtained with such bicarbonate solutions as are mentioned in the text. It may also be observed in this connection that in the observations made by Howell (which suggested this line of enquiry) the "5 to 5 solution" was frequently employed. It is therefore certain that the results obtained in the present research were not due to variations in the osmotic pressure of the solutions employed.

[4] Why, it might be asked, was not the Hürthle manometer sufficient to furnish the desired data? It is because in the course of these and other experiments it has been found that the readings of the Hürthle and of the maximum and minimum manometers do not always agree, may, in fact, differ markedly. The reason for these discrepancies is not at all clear to the author, but it is hoped that, after sufficient data have been collected, the cause may become apparent. In the meanwhile, for the determination of the systolic and diastolic pressures, the clumsier but more reliable apparatus, namely, the valved manometers, has been employed.

It was subsequently found that the system of tubes connecting the femoral artery with the mercury manometers possessed more extensibility than had at first been supposed. Investigations were therefore undertaken to determine the seriousness of this instrumental error. A comparison of the apparatus used in these experiments with a much more perfect one subsequently devised showed that of the results obtained with the former the mean pressures were quite accurate, but that the systolic was too low and the diastolic was too high. Although the absolute error was not inconsiderable, the relation of the systolic to the diastolic pressures was the same with both instruments. It follows, therefore, that the data obtained in this series of experiments are relatively correct.

The form of valve used was that known as the "Williams valve." For recording the movements of all these manometers a Hürthle kymographion was employed.

Secondary Experiments.—Upon several of the animals "secondary experiments" were performed. By this is meant that, after the dogs had been bled and infused and then allowed to remain undisturbed for a couple of hours, a second bleeding and infusion were undertaken. The data obtained from these secondary experiments have not been interspersed among those obtained from the primary experiments. The reason for this is obvious, namely, that the animals in these two series of experiments, the primary and the secondary, were under conditions which were not at all comparable. These secondary experiments proved to be of sufficient importance to merit attention, and, though few in number, gave results which were suggestive.

"*Principal Points.*"—Readings of the pulse rate and blood-pressures were made before, during, and usually for a considerable time after, the operations of bleeding and infusion. Just as temperatures are "charted" by the clinician, so in each of these experiments the rate and pressures were charted, so that the changes in these phenomena could easily be followed and studied. Obviously it would have been very inconvenient to reproduce many curves of this character, and, moreover, it would have been quite unnecessary to introduce as much detail as these curves contain into the present discussion. Hence certain "principal points" have been selected on each curve, and the data corresponding to them tabulated and compared. These principal points are: (1) immediately before bleeding, (2) soon after bleeding, but before infusion, (3) immediately after infusion, and (4) some time (about two hours) later. In the tables and discussion which follow, the data which correspond to these four points are, for the sake of brevity, designated as "before," "after," "immed.," and "late" pressures and rates respectively.

General Character of the Experiments.—The following table shows in a general way the character of each of the twelve primary and secondary experiments performed:

Date of experiment.	Weight of animal in kilogrammes.	Percentage of sodium bicarbonate in primary infusions.	Amount of blood withdrawn.[5]	Amount of infusion.[5]	Mean temperature of infusion.	Percentage of sodium bicarbonate in secondary infusion.	Amount of blood withdrawn.[5]	Amount of infusion.[5]	Mean temperature of infusion.
June 3	8.500	0	2.9 %	2.4 %	44°	—	—	—	—
" 7	4.500	0	3.3 %	4.4 %	38°	—	—	—	—
" 12	4.050	1	4.1 %	3.7 %	39°	[illegible]	2.4 %	2.4 %	40°
" 14	2.925	1	4.4 %	4.4 %	43°	—	—	—	—
" 18	7.650	1	4.2 %	1.9 %	42°	0	1.7 %	2.2 %	40°
" 24	8.685	0	2.8 %	1.7 %	48°	½	1.6 %	1.1 %	38°
" 27	8.000	0	4.0 %	2.4 %	43°	1	1.3 %	1.2 %	41°
" 30	4.950	½	3.3 %	3.0 %	42°	1	2.0 %	2.0 %	40°
July 1	6.750	0	3.9 %	2.9 %	40°	1	1.6 %	1.6 %	41°
" 3	5.400	½	4.2 %	1.8 %	43°	—	—	—	—
" 7	10.800	¼	4.0 %	2.3 %	48°	—	—	—	—
" 11	15.750	1/10	3.5 %	2.3 %	42°	—	—	—	—

THE EFFECT OF HÆMORRHAGE.

Pulse Rate in Primary Experiments.—As the result of hæmorrhage the pulse rate is usually much increased in the primary experiments. In these the amount of acceleration was very variable, the average increase being 29 per cent, the maximum being 96 per cent, while in only one case did the pulse rate remain unchanged.

The amount of acceleration did not bear any close relation to the severity of the hæmorrhage nor to the extent of the fall in the blood pressures. On the other hand, there is some evidence that *the amount of acceleration depends on the extent of the tonic activity of the vagus* at the time at which the bleeding was begun.

If, for instance, the normal rate be, say, 25 in 10 seconds, then the amount of the acceleration will be small; whereas in the case of a heart beating at the rate of, say, 13 in 10 seconds, there is a marked acceleration. This is shown in Table I, where it may be seen that in the first six experiments in which the acceleration is greater, the average normal heart rate is slow (18 in 10 seconds); while in the second six, in which the acceleration is less, the average normal heart rate is considerably greater (23 in 10 seconds). And, moreover, it may be observed that of the two hearts which showed the greatest acceleration,

[5] The amounts of blood withdrawn and of fluid infused are expressed in percentages of the body weight.

namely, Nos. 18 and 7u, one had much the slowest rate of all, and the other belonged to the group which comes next in order of slowness, namely, that composed of Nos. 7u, 30, and 12.[6]

Pulse Rate in Secondary Experiments.—The effect upon the pulse rate caused by the secondary bleedings is very different from that which occurs in the perfectly fresh animal. It has been shown that the acceleration due to hæmorrhage is probably due to the tonic action of the vagi. Now, at the time when the animal is about to undergo a second bleeding the heart is beating at about its maximum rate, so that no further increase in this rate would be expected. As a matter of fact, not only is the rate not increased, but it may even be diminished.[7]

A possible cause of this diminution is suggested by the investigations of Bergendal and Bergman. These authors have studied the effects upon the heart rate of rapid hæmorrhage from the carotid artery. They observed that as the bleeding proceeded, the preliminary acceleration gave place to a marked slowing. This slowing depended for its occurrence on the integrity of the vagi, and Bergendal and Bergman compared it to the similar phenomenon (likewise due to the action of the vagi) present in asphyxia, attributing both to the same cause, namely, the lack of oxygen. In the experiments of the author the effect of the primary hæmorrhage was to quicken the heart-beats. The infusion replaces the lost blood, but the oxygen carrying power of the circulating fluid remains low, so that when the secondary hæmorrhage took place the same result was obtained as if the primary hæmorrhage had been continued, i. e., the acceleration gave place to a slowing. Although the same result does not necessarily imply the same cause, yet the similarity is suggestive.

Blood Pressures in Primary and Secondary Experiments.—The abstraction of a considerable amount of blood results, of course, in a fall in the blood pressures which is more or less marked. Just before the end of the hæmorrhage, while the blood is still flowing from the artery, the lowest pressures are observed. On placing the clamp upon the artery, the pressures begin to rise

[6] These results accord with those obtained by Bergendal and Bergman (*Skand. Arch. f. Physiol.*, 1897, vii, 186). [7] See Table II.

	No. of experiment.	Normal pulse rate.	Pulse rate "before."	Pulse rate "after."	Acceleration.	Hæmorrhage.	Fall in pressures, i.e., "after" ÷ "before" × 100.			
							S	M	D	PP
TABLE I.	18		13	26	+ 96 %	4.2 %	43	58	52	34
Primary experiments arranged in order of greatest acceleration.	7u		18	28	+ 59 %	3.3 %	26	29	25	26
	1		25	35	+ 37 %	3.9 %	25	21	19	33
	12		17	24	+ 37 %	4.1 %	32	29	26	50
	30		17	22	+ 29 %	3.3 %	23	24	27	17
	3u		21	27	+ 27 %	2.9 %	47	52	47	47
	27		21	25	+ 20 %	4.0 %	21	21	21	20
	24		28	21	+ 15 %	2.8 %	23	31	38	8
	11		25	28	+ 12 %	3.5 %	23	22	25	21
	3y		20	22	+ 12 %	4.2 %	36	34	35	31
	14		20	21	+ 8 %	4.4 %	34	35	29	44
	7y		25	25	± 0 %	4.0 %	25	31	31	16
TABLE II.	1	25	32	34	+ 6 %	1.6 %	22	24	23	20
Secondary experiments arranged in order of greatest acceleration.	27	21	28	28	— 1 %	1.3 %	23	23	26	17
	30	17	22	21	— 5 %	2.0 %	22	31	35	12
	24	28	31	30	— 5 %	1.6 %	21	24	26	14
	18	13	33	28	— 16 %	1.7 %	26	25	29	20
	12	17	34	22	— 40 %	2.4 %	22	20	20	25

Pulse rates are the number of beats in 10 seconds.

Hæmorrhage is the blood withdrawn in percentage of the body weight.

Here as elsewhere S is systolic, M is mean, D is diastolic, and PP is pulse pressure.

"Before" and "after" are the "principal points" defined on page 4.

In the secondary experiments "normal rate" is rate "before" in the primary experiments.

slowly, owing doubtless to the increased peripheral resistance caused by the occlusion of the artery. This rise is slight (amounting to not more than 10 mm. Hg.), and the pressure then remains constant until the beginning of the infusion.[8]

That no relation exists between the amount of the fall in the pressure and the degree of the acceleration[9] is not especially surprising, but it seems rather remarkable that *the extent of the fall does not depend closely upon the amount of the blood withdrawn*

[8] It should be noted that the "principal point" designated "after" occurs after this rise has taken place and the pressure has become fairly constant.

[9] See page 4, and also Tables I and II.

(i. e., in proportion to the body weight), a fact which is made evident by the following tables (Tables III and IV):

	No. of experiment.	Fall in pressures, i. e., "after" ÷ "before" × 100. S	M	D	PP	Hæmorrhage.	Acceleration.
TABLE III.							
Primary experiments arranged in order of the amount of hæmorrhage.	24	23	31	38	8	2.8 %	+ 15 %
	3u	47	52	47	47	2.9 %	+ 27 %
	7u	26	29	25	26	3.3 %	+ 59 %
	30	23	24	27	17	3.3 %	+ 29 %
	11	23	22	25	21	3.5 %	+ 12 %
	1	25	21	19	33	3.9 %	+ 37 %
	27	21	21	21	20	4.0 %	+ 20 %
	7y	26	29	25	16	4.0 %	± 0 %
	12	32	29	26	50	4.1 %	+ 37 %
	18	43	51	52	34	4.2 %	+ 96 %
	3y	36	34	35	31	4.2 %	+ 12 %
	14	34	35	29	44	4.4 %	+ 08 %
TABLE IV.							
Secondary experiments arranged in order of the amount of hæmorrhage.	27	23	23	26	17	1.3 %	— 1 %
	24	21	24	26	14	1.6 %	— 5 %
	1	22	24	23	20	1.6 %	+ 6 %
	18	26	25	29	20	1.7 %	— 16 %
	30	22	31	35	12	2.0 %	— 5 %
	12	22	20	20	25	2.4 %	— 40 %

There are several possible causes for this want of agreement between the fall in pressure and the extent of the hæmorrhage. In the first place the amount of blood withdrawn is not an absolute indication of the severity of the hæmorrhage, for the amount of blood normally present in the dog varies somewhat in its relation to the body weight in different individuals. Consequently a hæmorrhage of, say, 4.5 per cent might be much more serious in one animal than in another. Secondly, the amount of the cardiac weakening due to the reduction in the blood supply of the coronary arteries varies in different hearts. Lastly, the amount of the peripheral constriction due to the anæmia might readily be supposed to vary in different individuals.

In order to analyse the relations of the systolic, mean, diastolic, and pulse pressures, both to the extent of the hæmorrhage and to one another, the author compiled a set of tables from the

data obtained in these experiments. From these tables, which for the sake of brevity have been omitted, certain negative conclusions were drawn which apply not only to the primary but also to the secondary experiments. These are the following:

(1) There is apparently no constant difference between the effects of hæmorrhage upon the systolic and upon the diastolic pressure. Either may be diminished more than the other, or they may be equally decreased.

(2) The relation of the decrease in the mean pressure to the fall in the systolic and diastolic pressures is very variable.

(3) The pulse pressure always shows a marked diminution, but this does not correspond in extent with the amount of the fall in mean pressure nor to the severity of the hæmorrhage.

It is, however, only proper to state that these negative results may be entirely due to those instrumental errors which with this apparatus always occur at low pressures, and to which reference will again be made.

THE EFFECT OF INFUSIONS.

It has seemed to the author that the presentation of the results of infusion could best be accomplished by means of a series of tables and charts, and, consequently, this method has been employed. The relation of the tables to the charts is such that the latter contain the gist of the former. Hence the tables and all that pertain to them exclusively have been printed in small type. That the tables have not been entirely omitted is owing to the fact that they show the various steps in the construction of the charts, and also because they furnish more exact data to such persons as may desire to peruse them.

As the phenomena studied in these experiments, namely, the pulse rate and the blood pressures, appear to be quite independent of each other, they will be considered separately.

Pulse Rate in Primary Experiments.—As any preliminary remarks would be superfluous, one may pass directly to a discussion of the tables and chart.

Table V shows the effect of hæmorrhage and infusion on the pulse rate, the rate having been determined at the four "principal points" already defined.

This table shows, of course, only the absolute changes in the rate, and hence it is difficult to compare the effects found in one experiment with those occurring in another. It was therefore thought desirable to modify the table by converting the absolute values into percentages. Consequently in Table VII the rates are given in percentages of the original rate which obtained before the bleeding was begun ("before"), while in Table IX the standard taken is the rate occurring after the hæmorrhage but before the infusion ("after").

The object in view is, however, to compare, not the effect of infusions on various individuals, but the effect of varying the composition of the fluid infused, and hence it is advantageous to have tables by means of which the average results gotten with the pure chloride may be compared with those obtained after the addition of bicarbonate. The results obtained by averaging Tables V, VII, and IX are seen in Tables VI, VIII, and X respectively.

No. of experiment.	Table V. Actual number of beats in periods of ten seconds.				Table VII. Relative number of beats per ten seconds in per cent of the "before" and "after" rates.				Table IX.		
	Before.	After.	Immed.	Late.	Before.	After.	Immed.	Late.	After.	Immed.	Late.
3u	21	27	27	—	100	127	130	—	100	192	—
7u	18	28	29	—	100	159	164	—	100	103	—
24	28	31	33	31	100	113	119	112	100	102	112
27	21	25	25	28	100	120	142	135	100	85	91
1	25	35	30	32	100	137	118	125	100	85	91
11	25	28	26	27	100	112	104	108	100	92	96
7y	25	25	24	36	100	100	94	141	100	94	141
30	17	22	23	22	100	129	135	129	100	104	100
3y	20	22	26	29	100	112	132	147	100	117	131
14	20	22	26	—	100	108	131	—	100	121	—
12	17	24	22	34	100	138	128	194	100	93	142
18	13	26	15	33	100	196	111	244	100	51	124
	Table VI. (Averages.)				Table VIII. (Averages.)				Table X. (Averages.)		
0	22	29	30	31	100	132	130	125	100	99	102
$\frac{1}{10}$	25	28	26	27	100	112	112	108	100	92	96
$\frac{1}{4}$	25	25	24	36	100	100	94	141	100	94	141
$\frac{1}{2}$	18	22	25	26	100	120	133	128	100	110	115
1	17	24	21	33	100	147	123	220	100	90	132

A perusal of the foregoing tables and of chart I. shows that there is no very evident connection between the presence of sodium

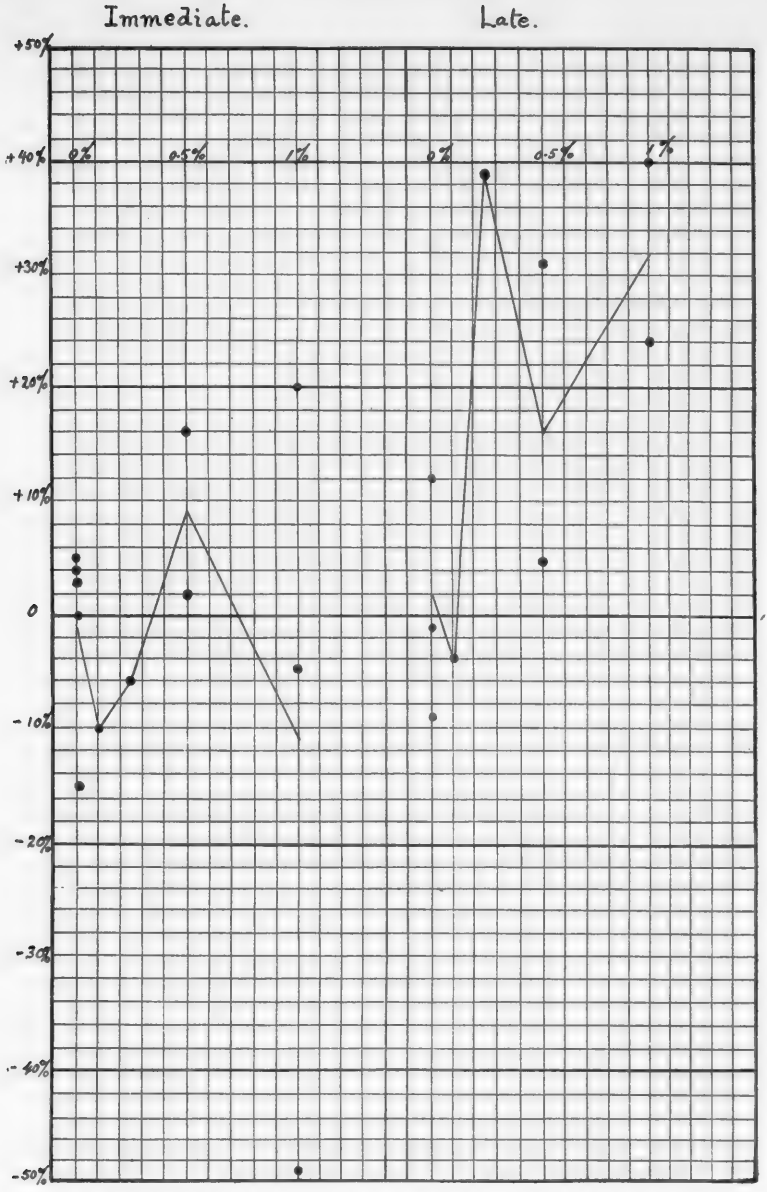


Chart I. Primary experiments

This chart, constructed from the data contained in Table X, shows the effect upon the pulse rate in the primary experiments of increasing the amount of bicarbonate infused. The percentage of sodium bicarbonate which was added to the solutions of 0.8 % chloride is indicated along the abscissa, while the percentage of acceleration, whether positive or negative, is given along the ordinate. The dots represent the results of single experiments, and the averages of these experiments are connected by means of a line. The terms "immediate" and "late" have already been defined.

bicarbonate and the rate at which the heart beats immediately after the infusion. On the other hand, there seems to be some difference with regard to the late effects. In this respect both tables and charts show that when bicarbonate is used, the heart rate continues to increase gradually and beats rapidly for some time.

Pulse Rate in Secondary Experiments.—The nature of what the author has chosen to call "secondary experiments" has already been explained. The results obtained in these experiments have been tabulated and charted in a manner precisely similar to that employed in the case of the primary experiments and will now be presented.

				Table XI.				Table XII.				Table XIII.		
No. of experiment.	Percentage of bicarbonate in primary infusion;	in secondary infusion.	Original rate.	Before.	After.	Immed.	Late.	Before	After.	Immed.	Late.	After.	Immed.	Late.
12	1	0	17	34	22	26	—	100	66	78	—	100	118	—
18	1	0	13	33	29	28	32	100	87	85	97	100	98	113
27	0	1	21	28	28	30	—	100	99	106	—	100	107	—
19	0	1	25	32	34	32	34	100	106	100	106	100	94	100
24	0	½	28	31	30	31	31	100	96	99	100	100	103	104
30	½	1	17	22	18	19	19	100	82	86	86	100	105	105
				(Averages.)				(Averages.)				(Averages.)		
	1	0	15	33	25	27	32	100	72	81	97	100	107	114
	0	1	23	30	31	31	34	100	100	97	96	100	100	102
	0	½	28	31	30	31	31	100	96	99	100	100	103	104
	½	1	17	22	18	19	19	100	82	86	86	100	105	105

The effect of infusion upon the heart rate, which is observed in these secondary experiments, does not at all resemble that obtained at the time of the primary experiments. In the case of the primary experiments it will be remembered, the addition of sodium bicarbonate to the infused liquid had for its fairly constant result an increase in the amount of the cardiac acceleration, the rate increasing gradually and remaining high for a considerable period of time. There is no such increase in the case of the secondary experiments.

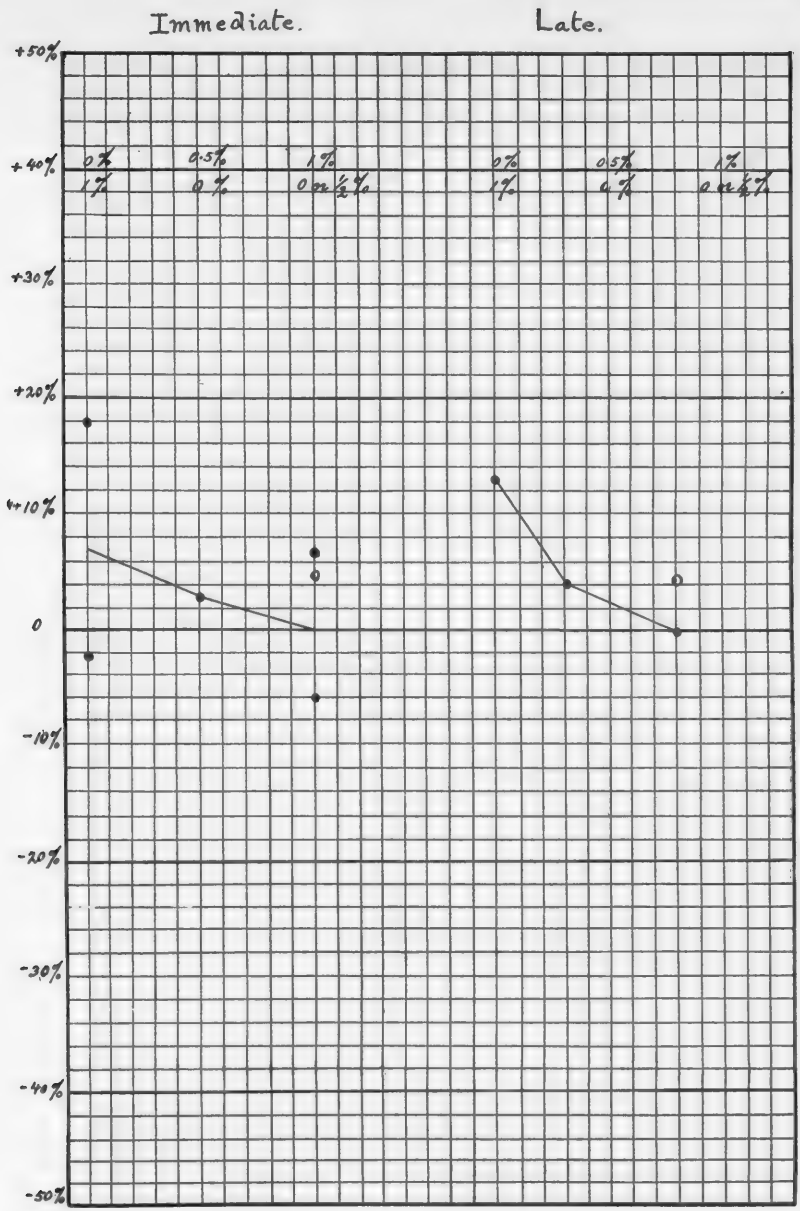


Chart. II. Secondary experiments.

This chart, compiled from the data contained in the latter part of Table XIII, shows for the secondary experiments what Chart I has already shown for the primary experiments. The explanation accompanying Chart I will serve to elucidate this one also. It may be added, however, that of the two numbers corresponding to each group of experiments, the upper gives the percentage of bicarbonate present in the secondary experiment, while the lower indicates the strength of bicarbonate infused in the preceding primary experiments performed upon the same animals. Since 1 % bicarbonate in some cases followed an infusion containing no bicarbonate, and in another case an infusion of ½ % of the same substance, separate designations have been employed, ● having been used for the former and o for the latter.

At the beginning of the secondary experiments the heart was beating at about its maximum rate. The effect of hæmorrhage is then (as has already been pointed out) to slow the rate. The immediate effect of the infusion (as shown by the tables and chart) is variable and inconsiderable. Thus the rate may be increased or decreased, or may suffer almost no modification whatsoever. Subsequently there is a slow increase until the heart rate approximates that which had originally obtained at the beginning of the secondary experiment.

Blood Pressures.—The effect of the infusions upon the blood pressures (systolic, mean, diastolic, and pulse pressure) can be presented most clearly by means of the method already employed, namely, by means of a series of tables and charts.

Primary Experiments.—In Table XIV are given the blood pressures as actually measured (mm. Hg.) in each of the primary experiments. This is immediately followed by Table XV, which shows the average pressures [10] compiled from those experiments in which animals were infused with liquids of the same

[10] In making up these averages the following source of error had to be borne in mind, namely, that in experiments 3u, 7u, and 14 no late readings were obtained. From this it follows that in some cases the averages of the "before" pressures are hardly comparable with those of the "late" pressures, and hence a direct comparison should be avoided. For example, in the case of those experiments in which pure chloride was employed, five factors enter into the average of the pressures "before," while the average of the "late" pressures is derived from only three factors. To avoid this source of error, the author resorted to a simple manœuvre in proportion, of which the following may serve as an example:

Average systolic pressure "before" in 3u, 7u, 24, 27, and 1 = 164
" " " " " 24, 27, and 1 = 173
" " " "late" " 24, 27, and 1 = 136

Hence $\frac{x}{173} = \frac{136}{164}$, or $x = 129$.

This is as much as saying that if the sum of the "late" results of the three experiments in which the "late" readings were recorded bore the same ratio to the sum of the corresponding pressures "before," as the "late" results of all five experiments (had they been obtained) to the sum of the corresponding "before" pressures, then 129 is a more correct estimate of the late pressures than is 136. Although this assumption is somewhat gratuitous, it is thought that by making such a correction the figures obtained might be less likely to produce a false impression and to lie nearer the truth than would values obtained by simply averaging. Consequently, this corrective procedure has been adopted wherever it has been deemed expedient.

Table XIV.

Actual pressures as measured in mm. Hg.

Bc %	No.	Before. S	M	D	PP	PP%	After. S	M	D	PP	PP%	Immed. S	M	D	PP	PP%	Late. S	M	D	PP	PP%
0	3u	140	85	63	77	55	66	44	30	36	54	125	76	41	84	67	—	—	—	—	—
0	7u	160	101	87	73	46	41	29	22	19	46	132	73	48	84	64	—	—	—	—	—
0	24	155	103	79	76	49	36	32	30	6	16	130	75	64	66	51	135	96	78	57	42
0	27	174	111	95	79	45	36	23	20	16	44	133	77	60	73	55	140	100	83	57	41
0	1	190	120	106	84	44	48	25	20	28	58	125	73	58	67	54	135	103	85	50	37
$\frac{1}{10}$	11	185	116	88	95	52	42	26	22	20	47	170	95	75	95	56	152	98	72	80	52
$\frac{1}{4}$	7y	146	107	90	56	38	37	33	28	9	24	138	109	90	48	35	125	91	70	55	44
$\frac{1}{2}$	30	150	102	84	66	44	34	25	23	11	32	155	101	85	70	45	120	67	52	68	57
$\frac{1}{2}$	3y	170	113	100	70	41	61	39	35	26	43	178	101	83	95	53	115	75	62	53	46
1	14	140	104	95	45	32	48	36	28	20	42	150	129	119	31	21	—	—	—	—	—
1	12	140	115	100	40	28	46	33	26	20	43	154	128	116	38	25	105	82	65	40	38
1	18	215	127	116	99	46	94	65	60	34	36	190	141	138	52	27	160	122	100	60	37
Averages:		164	109	92																	

Table XV.

(Averages.)

Bc %	Before. S	M	D	PP	PP%	After. S	M	D	PP	PP%	Immed. S	M	D	PP	PP%	Late. S	M	D	PP	PP%
0	164	104	86	78	48	45	31	24	21	44	129	75	54	75	58	129	93	75	54	57
$\frac{1}{10}$ and $\frac{1}{4}$	165	111	89	75	45	39	29	25	14	35	154	102	82	71	45	138	94	71	67	48
$\frac{1}{2}$	160	107	92	68	42	47	32	29	18	37	166	101	84	82	49	117	71	57	60	61
1	165	115	104	61	35	63	45	38	25	40	164	133	124	40	24	123	96	79	44	37

Bc % is the percentage of sodium bicarbonate present in the solution infused. No. is number of the experiment. S, M, and D = systolic, mean, and diastolic pressures respectively. PP is pulse pressure, i. e., S minus D. PP % is percentage pulse pressure, i. e., D divided by S multiplied by 100. "Before," etc. = the "principal points."

composition.[11] Since, however, the normal pressures in individual animals varied considerably, Tables XIV and XV, for example, give little conception of the relative changes which took place. Consequently, from the data furnished by these tables, two others, Tables XVI and XVII, have been constructed, which present the pressure changes in percentages of the pressures "before" hæmorrhage.

			After.				Immed.				Late.			
	Bc %	No.	S	M	D	P	S	M	D	P	S	M	D	P
TABLE XVI.	0	3u	47	52	47	47	89	89	65	109	—	—	—	—
	0	7u	26	29	25	26	82	72	55	115	—	—	—	—
	0	24	23	31	38	3	84	73	81	87	87	93	99	75
	0	27	21	21	21	20	76	69	63	92	80	91	87	72
	0	II	25	21	19	33	65	61	55	79	71	86	80	59
	$\frac{1}{10}$	11	23	22	25	21	92	82	85	100	82	84	82	84
	$\frac{1}{4}$	7y	25	31	31	16	94	102	100	86	85	85	77	98
	$\frac{1}{2}$	30	23	24	27	17	103	99	101	106	80	66	62	103
	$\frac{1}{2}$	3y	36	34	35	31	105	89	83	136	68	66	62	75
	1	14	34	35	29	44	107	124	125	61	—	—	—	—
	1	12	32	29	26	50	110	111	116	95	75	71	65	100
	1	18	43	51	52	34	88	111	119	52	74	96	86	61
TABLE XVII. (Averages.)	0		28	31	30	27	79	73	64	96	79	89	87	69
	$\frac{1}{10}$ and $\frac{1}{4}$		24	26	28	18	93	92	92	93	83	84	79	91
	$\frac{1}{2}$		29	29	31	24	104	94	92	121	74	66	62	89
	1		36	38	36	42	102	115	120	69	84	76	83	54

Since the "before" pressures which correspond to the values given above are in every case equal to 100, they have been omitted from the table.

At first sight it might seem that it would have been more logical to give a table in which the pressures "after" hæmorrhage, but before infusion, had been taken as a standard, while the remaining pressures were presented as percentages of that standard. When, however it is remembered[12] that the smaller the pulse pressure and the lower the remaining pressures, the more inaccurate are the readings of the maximum-minimum manometer, it can readily be inferred that such a table would be worse than valueless.

Naturally the facts embodied in these tables can be grasped more easily when presented graphically, as in Chart III. In constructing this chart, showing as it does, all the blood pressures simultaneously, a difficulty was encountered, namely, the fact that one is here dealing with three independent quantities

[11] It should be noted that the two experiments in which the amounts of bicarbonate infused were $\frac{1}{4}$ and $\frac{1}{10}$ % respectively have also been grouped together.

[12] See above, p. 9.

(systolic, mean, and diastolic pressures), each of which should be represented under standard conditions by the figure 100. To avoid the confusion which would occur in the construction of a table requiring the use of three separate scales, the author resorted to the manœuvre of reducing all the values given in Tables XIV and XV to a common standard. This was accomplished in the following manner:

Consider Table XIV; the average normal systolic, mean, and diastolic pressures of the twelve experiments "before" hæmorrhage are respectively 164, 109, and 92. All the other values given in this table may be reduced to this common standard, thus:

In Experiment 3u systolic pressure "after" is 66 mm. Hg.

In Experiment 3u systolic pressure "before" is 140 mm. Hg.

Find the value of 3u syst. "after" when the syst. "before" is 164.

$$\frac{x}{164} = \frac{66}{140} \therefore x = \frac{66 \times 164}{140} = 77$$

This is the value of 3u syst. "after" given in Table XVIII, and this method was employed in obtaining all of the values presented in Tables XVIII and XIX. In Table XIX the averages for Table XVIII are given. These two tables are, however, to be regarded only as a step in the construction of Chart III, and are inserted here mainly for the sake of completeness.

			After.			Immed.			Late.		
	Bc %	No.	S	M	D	S	M	D	S	M	D
TABLE XVIII.	0	3u	77	56	44	146	97	60	—	—	—
	0	7u	41	31	23	135	79	51	—	—	—
	0	24	38	34	35	138	79	74	143	101	90
	0	27	33	22	19	125	75	58	132	98	80
	0	1	41	23	17	107	60	50	116	94	74
	$\frac{1}{10}$	11	37	24	23	150	89	78	134	92	75
	$\frac{1}{4}$	7y	41	37	28	154	111	92	140	93	71
	$\frac{1}{2}$	30	37	27	25	169	108	93	131	72	57
	$\frac{1}{2}$	3y	58	37	32	178	97	76	110	72	57
	1	14	56	38	27	175	135	115	—	—	—
	1	12	54	31	24	180	122	107	123	78	60
	1	18	71	55	47	144	120	109	122	104	79
TABLE XIX. (Averages.)	0		46	33	28	130	78	59	130	98	81
	$\frac{1}{10}$ and $\frac{1}{4}$		39	30	25	152	100	85	137	92	73
	$\frac{1}{2}$		47	32	28	170	102	84	120	72	57
	1		60	41	33	166	126	110	122	91	69

Since the "before" pressures which correspond to the values given above are in every case S 164, M 109, and D 92, they have been omitted from the table.

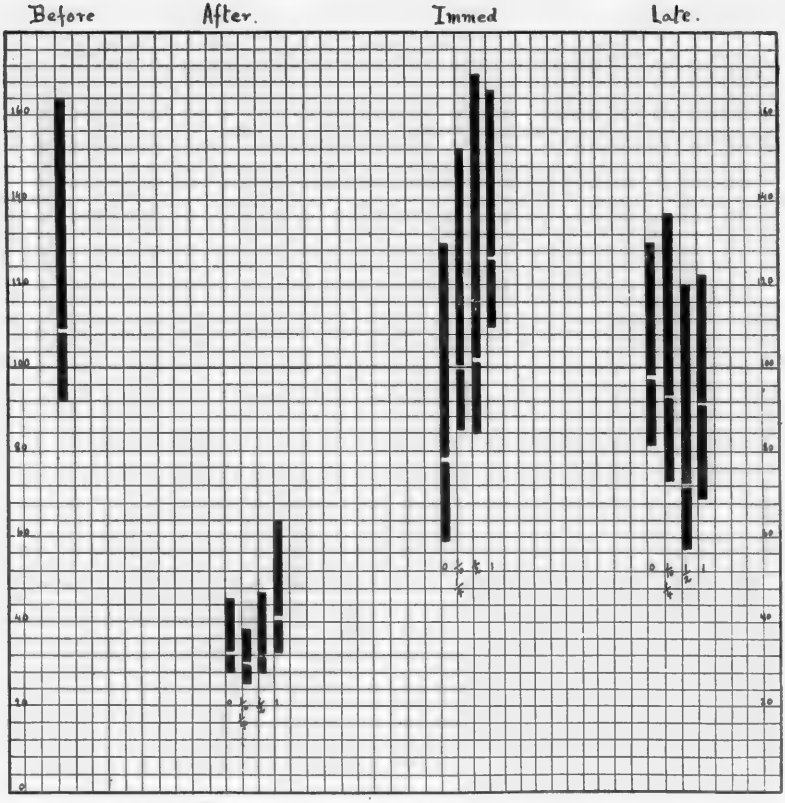


Chart III. Primary experiments.

This chart shows the effects of infusion upon the "blood pressures" in the twelve "primary" experiments. The dark vertical lines represent the "blood pressures," the top of the lines being the systolic, the bottom the diastolic, pressure; the point at which the line is broken indicates the mean pressure, while each line as a whole represents a pulse pressure. The terms "before," "after," etc., are the "principal points" which have already been defined.

The first vertical line represents the average of the pressures in all twelve experiments immediately "before hæmorrhage." The second vertical line represents the condition of the pressures "after" bleeding in those dogs which were afterwards infused with pure sodium chloride, or, in other words, with a solution containing o % of sodium bicarbonate, as indicated by the zero placed below the line. The sixth and tenth lines represent the pressure conditions obtaining in the same series of animals "immediately" after the infusion and some hours "later" respectively. The absence of bicarbonate from the liquid infused in these cases is indicated by the o placed beneath the vertical lines in question.

The third, seventh, eleventh, and fifteenth lines show the corresponding values for the experiments in which $\frac{1}{10}$ % and $\frac{1}{4}$ % bicarbonate were added to the o.8 % chloride. The signification of the remaining lines can easily be inferred.

	Bc. % Prim.	Bc. % Sec.	No.	Before. S	M	D	PP	PP%	After. S	M	D	PP	PP%	Immed. S	M	D	PP	PP%	Late. S	M	D	PP	PP%
TABLE XX.	1	0	12	105	82	65	40	38	23	16	13	10	43	122	71	45	77	63	—	—	—	—	—
	1	0	18	160	122	100	60	37	41	31	29	12	29	160	109	85	75	46	—	—	—	—	—
	0	1	27	140	100	83	57	41	32	23	22	10	31	190	102	70	120	60	—	—	—	—	—
	0	1	1y	135	103	85	50	37	30	25	20	10	33	153	136	113	40	26	86	63	54	32	37
	0	½	24	135	96	78	57	42	28	23	20	8	28	170	99	67	103	60	110	70	48	62	56
	½	1	30	120	67	52	68	57	26	21	18	8	30	100	52	45	55	55	89	46	38	51	57
	Averages:			132	95	77																	
TABLE XXI. (Averages.)	1	0		132	102	82	50	38	32	23	21	11	34	141	90	65	76	54	—	—	—	—	—
	0	1		137	101	84	53	39	31	24	21	10	32	171	119	91	80	46	87	64	54	32	37
	0	½		135	96	78	57	42	28	23	20	8	28	170	99	67	103	60	110	70	48	62	56
	½	1		120	67	52	68	57	26	21	18	8	30	100	52	45	55	55	89	46	38	51	57

Secondary Experiments.—The next series of tables deals with the secondary experiments. These tables resemble in form those already given, and their contents may be summarized as follows:

Table XX contains actual pressures in mm. Hg., followed by Table XXI, containing the averages of the preceding. These two correspond to Tables XIV and XV of the primary experiments.

Table XXII contains percentage pressures, the pressures "before" hæmorrhage being represented by 100. Table XXIII presents the corresponding averages. These two are similar to Tables XVI and XVII of the primary experiments.

Table XXIV contains relative pressures reduced to the standard S being 132, M, 95, and D, 77, these being the values of the average pressures "before" hæmorrhage in all the secondary experiments, while Table XXV gives the

				After.				Immed.				Late.			
	Bc. % Prim.	Bc. % Sec.	No.	S	M	D	PP	S	M	D	PP	S	M	D	PP
TABLE XXII.	1	0	12	22	20	20	25	116	86	69	192	—	—	—	—
	1	0	18	26	25	29	20	100	89	85	125	—	—	—	—
	0	1	27	23	23	26	17	136	120	84	210	—	—	—	—
	0	1	1y	22	24	23	20	113	133	132	80	64	61	63	64
	0	½	24	21	24	26	14	126	103	86	181	81	72	61	109
	½	1	30	22	31	25	12	85	78	86	81	74	69	73	75
	(Averages.)														
TABLE XXIII.	1	0		24	22	24	22	108	87	77	158	—	—	—	—
	0	1		22	23	24	18	124	126	108	145	45	60	67	59
	0	½		21	24	26	14	126	103	86	181	81	72	61	109
	½	1		22	31	35	12	83	78	86	81	74	69	73	75

Since the "before" pressures which correspond to the values given above are in every case equal to 100, they have been omitted from the tables.

				After.				Immed.				Late.			
	Bc. % Prim.	Bc. % Sec.	No.	S	M	D	PP	S	M	D	PP	S	M	D	PP
	1	0	12	29	18	15	14	153	82	53	100	—	—	—	—
TABLE XXIV.	1	0	18	34	24	22	12	132	85	65	67	—	—	—	—
	0	1	27	30	22	20	10	179	97	65	114	—	—	—	—
	0	1	1y	29	23	18	11	149	125	102	47	84	58	49	35
	0	½	24	27	23	20	7	166	98	66	100	107	69	47	60
	½	1	30	29	30	27	2	110	74	67	43	91	65	56	35
	(Averages.)														
TABLE XXV.	1	0		31	21	18	13	142	83	59	83	—	—	—	—
	0	1		29	22	19	10	164	111	83	81	84	58	49	35
	0	½		27	23	20	7	166	98	66	100	107	69	47	60
	½	1		29	30	27	2	110	74	67	43	91	65	56	35

The "before" pressures which have been omitted from these tables are in every case S 132, M 95, D 77, and PP 55.

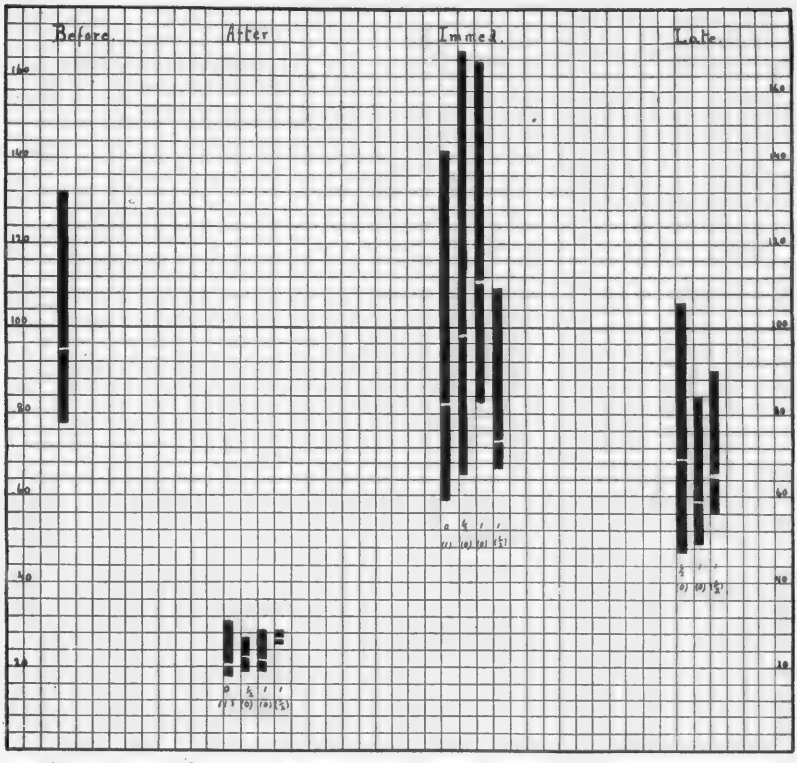


Chart IV. Secondary experiments.

This chart shows the effects of infusion upon the blood "pressures" in the six "secondary" experiments. The explanation accompanying the preceding chart (Chart III) will serve to elucidate this one also. It may be added, however, that, of the two numbers which are placed at the foot of each vertical line, the upper one corresponds to the secondary infusion, while the lower one (placed in parentheses) indicates the nature of the primary infusion. Thus $\frac{\frac{1}{2}}{(0)}$ means that the group of animals of which the pressures are indicated by the line above the symbols received first an infusion of 0.8 % sodium chloride, and later an infusion of 0.8 % sodium chloride + 0.5 % sodium bicarbonate.

averages. These correspond to Tables XVIII and XIX of the primary experiments. It is from the data given in Table XXV that Chart IV has been constructed.

Relative Efficiency of Different Infusions.—On inspection of Table I it may be seen that the chloride and bicarbonate were not infused in equal quantities, the amount of the latter being less (sometimes very much less) than that of the former. If, then, it be desired to compare the chloride and the bicarbonate with respect to their powers of raising the blood pressure, the fact that it requires less of the latter than of the former to bring about the same result should not be forgotten.

In estimating the power of a substance to raise the blood pressure, it should be borne in mind that within limits the extent of the rise in the pressure will depend upon the amount of the fluid infused. Moreover, other things being equal the efficiency of a given amount of an infused liquid will depend upon the amount of blood previously removed. With the former (the amount of the fluid infused) the efficiency will vary directly, with the latter (the amount of the hæmorrhage) inversely.

Thus if E = the efficiency, I = the amount of infusion,
H = the amount of hæmorrhage and K be a constant,

then $\frac{E}{K} = \frac{I}{H}$ $\therefore$ $\frac{E\,H}{I} = K$.

Strictly speaking, E should be the ratio of the pressures $\frac{\text{"Immed." or "Late"}}{\text{"After"}}$ i. e., the rise in pressure due to infusion. The author is, however, loath to employ the "after" pressures as divisors, since the determination of these pressures almost certainly involves a comparatively large amount of experimental error,[13] so that he has preferred to take the ratios $\frac{\text{"Immed." or "Late"}}{\text{"Before"}}$ as indicating the efficiency of the liquid in restoring the blood pressures to their normal levels. Consequently the equation, $\frac{\text{"Immed." or "Late"}}{\text{"Before"}} \times \frac{H}{I} = K$ may be considered as equivalent to the equation $\frac{E\,H}{I} = K$.

[13] See pages 9 and 16.

In Table XXVI are seen the values of the ratio $\frac{H}{I}$ i. e., $\frac{\text{Hæmorrhage}}{\text{Infusion}}$ and here also are to be found the values of the expression $\frac{\text{Immed. and Late}}{\text{Before}} \times \frac{H}{I}$. Table XXVII contains the corresponding averages.

	Character of the infusion.	No. of experiment.	$\frac{H}{I}$	Relative efficiency. Immed. S	Immed. M	Immed. D	Late. S	Late. M	Late. D
TABLE XXVI. Primary experiments.	0	3u	120	107	107	78	—	—	—
	0	7u	75	61	53	41	—	—	—
	0	24	164	138	120	133	143	152	162
	0	27	166	126	114	104	133	151	144
	0	1	134	87	82	74	95	115	107
	$\frac{1}{10}$	11	152	138	123	127	123	126	123
	$\frac{1}{4}$	7y	173	163	176	173	147	147	133
	$\frac{1}{2}$	30	110	113	109	111	88	73	68
	$\frac{1}{2}$	3y	233	245	207	193	158	154	144
	1	14	100	107	124	125	—	—	—
	1	12	110	122	123	129	83	78	72
	1	18	221	194	245	263	163	212	190
TABLE XXVII (Averages.)	0		132	104	96	84	129	142	137
	$\frac{1}{10}$ and $\frac{1}{4}$		162	151	149	149	134	136	128
	$\frac{1}{2}$		171	178	161	157	126	113	106
	1		143	146	164	172	123	145	131

These data have also been determined for the secondary experiments and are presented in Tables XXVIII and XXIX.[14]

[14] The chart corresponding to Table XXIX has been omitted for the sake of brevity.

	Primary infusion.	Secondary infusion.	No. of experiment.	$\frac{H}{I}$.	Relative efficiency. Immed. S	Immed. M	Immed. D	Late. S	Late. M	Late. S
TABLE XXVIII.					S	M	D	S	M	S
	1	0	12	100	116	86	69	—	—	—
	1	0	18	130	129	115	109	—	—	—
	0	1	27	90	125	110	77	—	—	—
	0	1	1y	100	113	133	132	64	61	63
	0	½	24	70	87	71	59	56	50	42
	½	1	30	100	83	78	86	74	69	73
TABLE XXIX.	1	0		115	122	100	89	—	—	—
	0	1		95	119	121	104	64	61	63
(Averages.)	0	½		70	87	71	59	56	50	42
	½	1		100	83	78	86	74	69	73

It can readily be seen from the foregoing tables that K ($\frac{E H}{I}$ = K) is by no means a constant quantity, but that it varies greatly, due probably to individual differences in the animals. This fact renders the danger of relying on average figures even greater than usual, and consequently it has been deemed advisable to present a chart (Chart V) containing not only the averages, but likewise the data from all the experiments.

Experiments 12 and 1y.—Up to the present the fact has not been emphasized that the secondary experiments were performed upon the same animals as the primary experiments. If all the experiments had been performed upon separate animals, then the phenomena which have been attributed to the effect of the bicarbonate might have been due to the idiosyncracies of the individual animals. The probability of this explanation decreases, of course, as the number of the experiments increases, but the crucial test would be obtained by performing a double experiment on two dogs, injecting into A chloride and then bicarbonate, while injecting into B the same solutions in the reverse order.

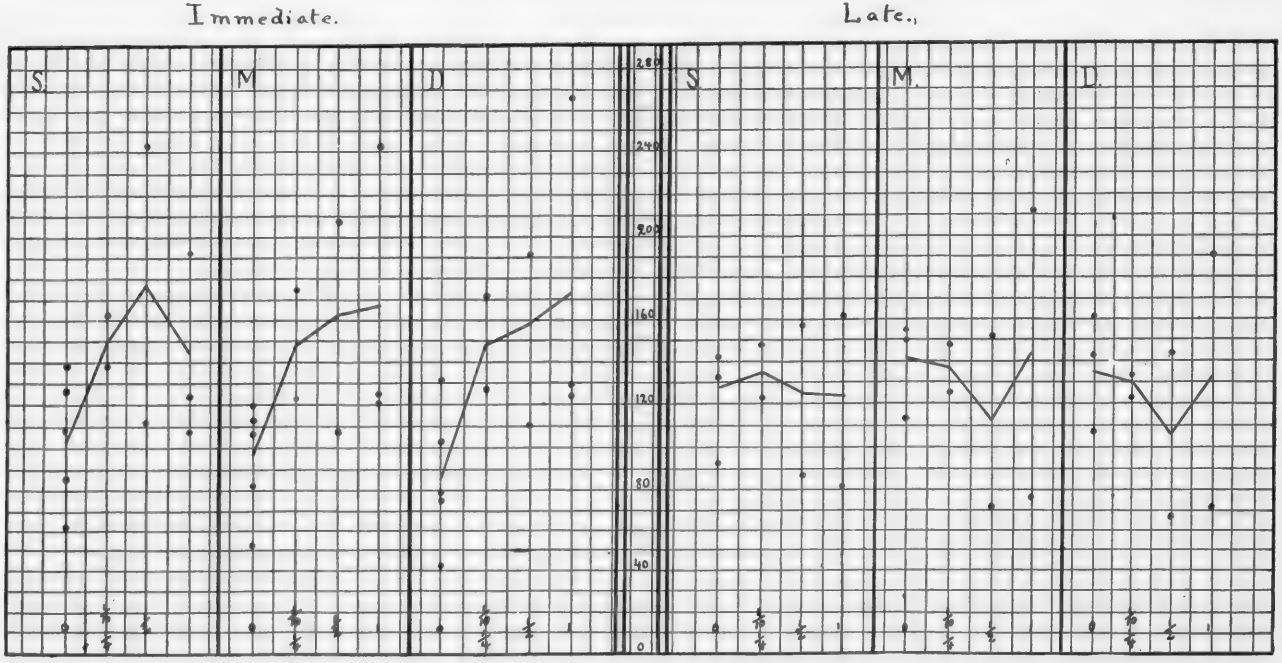
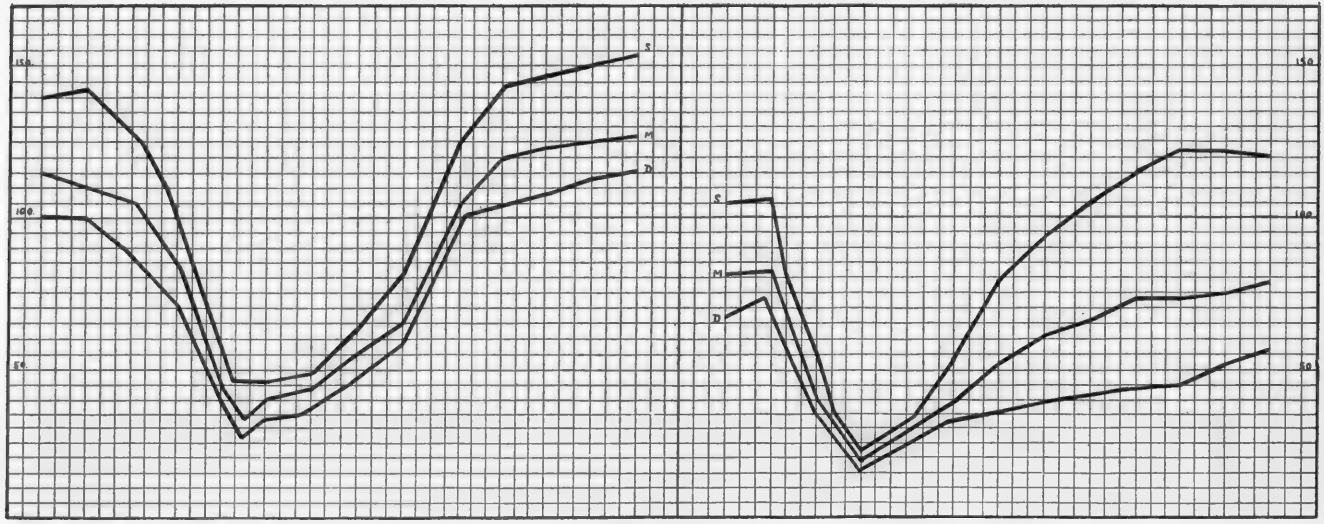


Chart v.

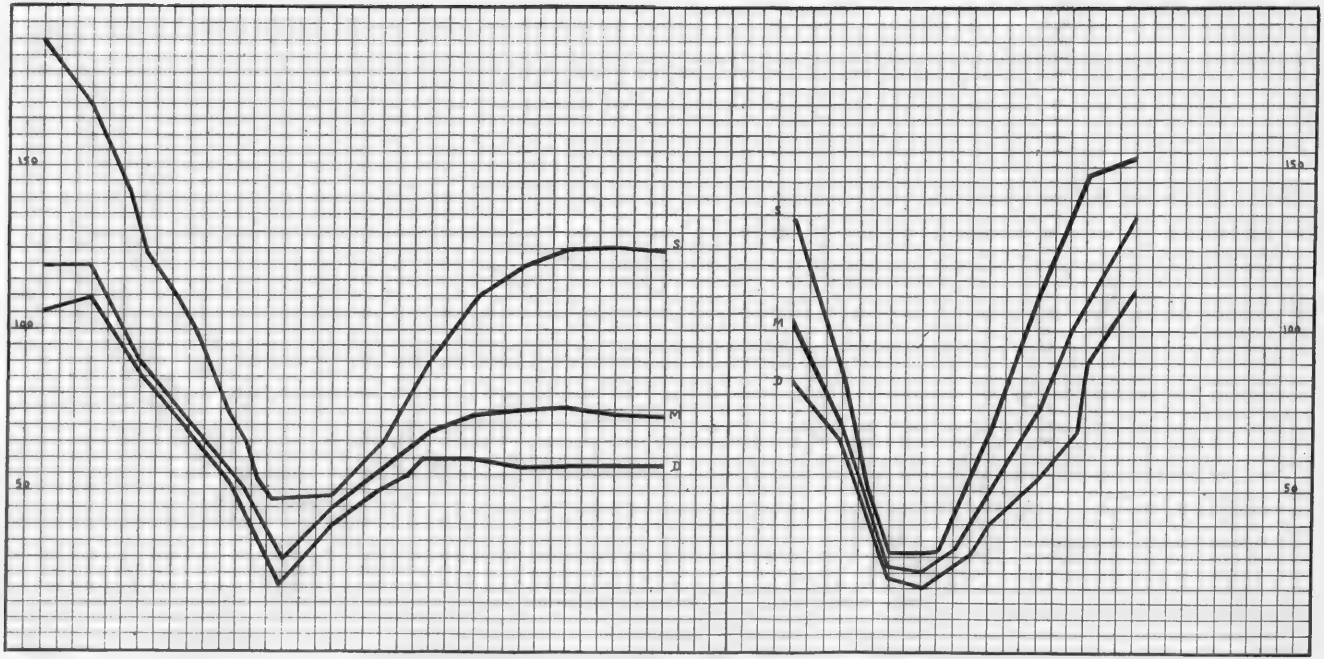
This chart shows that the efficiency of the solution in raising the blood-pressure is increased by increasing the amount of bicarbonate of sodium. S, M, and D signify the systolic, mean, and diastolic pressures respectively. The dots represent the results of single experiments while the averages of these experiments are connected by a line. The percentages of bicarbonate present in the various solutions are indicated along the abscissa, and the units of efficiency are marked off along the ordinate.



No. 12. Primary, 1%. Chart VI. Secondary, 0%.

This chart shows the results of two experiments performed on dog No. 12. In the primary experiment the amount of blood withdrawn was 4.1 % of the body weight. After this the animal was infused with a solution of 0.8 % sodium chloride + 1.0 % sodium bicarbonate, amounting to 3.7 % of the body weight. In the secondary experiment the amount of blood withdrawn was 2.4 % of the body weight, while the infusion contained only 0.8 % sodium chloride and amounted to 2.4 % of the body weight.

The line designated by the letter "S" represents the systolic, "M" the mean, "D" the diastolic pressure, while the distance between S and D shows the pulse pressure.



No. 1y. Primary, 0%. Chart VII. Secondary, 1%.

This chart should be compared with the preceding (Chart VI). It shows the results of two experiments performed on dog No. 1y. In the primary experiment the amount of blood withdrawn was 3.9 % of the body weight, and the infusion was one of 0.8 % sodium chloride, amounting to 2.9 % of the body weight. In the secondary experiment the amount of blood withdrawn was 1.6 % and the amount of the infusion also 1.6 %, the latter containing 0.8 % sodium chloride + 1.0 % sodium bicarbonate.

Such an experiment has actually been performed as is seen when both the primary and secondary experiments performed upon dogs 12 and 1y are taken into consideration. The charts obtained in these experiments, after having been somewhat simplified, are presented as Charts VI and VII. on pages 26 and 27.

Conclusions.—After a careful study of the data presented above, the author has reached the conclusions which are given below and which, if legitimate, should be capable of verification by any one who will compare them with the foregoing tables and charts.

(1) If sodium chloride be infused after severe hæmorrhage, the immediate effect is an increase of the blood pressures. Of these the systolic pressure is the most nearly restored to normal, next in order of completeness of the restoration comes the mean, and lastly the diastolic, these pressures being 79, 73, and 64 per cent, respectively, of the original pressures. Since the increase of the systolic pressure is greater than that of the diastolic, the pulse pressure remains large and is, in fact, nearly normal (96 per cent).

(2) If, now, to solutions of the chloride an increasing percentage of sodium bicarbonate be added, the rise in the pressures is markedly increased.

(3) The rise in the systolic pressure may reach a maximum of 115 per cent of its original value. This maximum occurs with the addition of ½ per cent bicarbonate to the chloride, and a further addition of carbonate (up to 1 per cent) is not accompanied by a further increase in the systolic pressure.

(4) The rise of the mean and of the diastolic pressure differs from that of the systolic pressure in that the maximum is not reached with a solution of ½ per cent of bicarbonate. The maxima were, for the mean, 115, and, for the diastolic pressure, 120 per cent of the original ("before") pressures, and were obtained with a solution containing 1 per cent of the bicarbonate, i. e., the strongest solution used.

(5) With a concentration of bicarbonate which is not greater than ½ per cent, the pulse pressure is increased considerably (121 per cent) above the normal. Owing to the fact that the

maximum systolic pressure is reached with a lower concentration than is the maximum diastolic, an increase in the amount of bicarbonate present in the infusion fluid to 1 per cent is accompanied by a marked diminution of the pulse pressure (69 per cent of the original value).

If, two hours after the infusion, the pressures be again observed, it is found that:

(6) In those experiments in which the chloride alone was used, the mean and diastolic pressures have, since the time of the infusion, gradually risen, although the systolic pressure has remained unchanged.

(7) On the other hand, when the carbonate is used all the pressures decrease considerably, so that the systolic is now no greater than it is with infusions of pure chloride, while the mean and diastolic pressures have become less, sometimes considerably less.

(8) In all cases the pulse pressure is considerably diminished. With pure chloride and with 1 per cent bicarbonate the diminution is greater (69 and 54 per cent of the original value respectively), while with a concentration of bicarbonate not greater than $\frac{1}{2}$ per cent the diminution is less (averaging 90 per cent).

Allowing for the fact that in the experiments with pure chloride the amount of fluid infused was almost always greater than in those experiments in which the bicarbonate was added, and when, in this connection, Chart V is carefully examined it is seen that:

(9) The bicarbonate appears still more efficient than the pure chloride, since the rise which is immediately brought about by the former is considerably the greater, while in the late effects the two solutions differ but little.

Although the secondary expermets arine somewhat wanting both in completeness and in numbers, nevertheless the conclusions to which they point are not without interest. These conclusions are the following:

(10) In the secondary experiments the results obtained by

infusions of pure chloride and of solutions containing bicarbonate are strikingly similar to those already described in the primary experiments.

(11) In the late effects, however, some difference is seen. Here while the diastolic pressures are nearly equal, whether the ½ or 1 per cent of bicarbonate be added, still both the mean and systolic and consequently the pulse pressure are considerably greater in the former (½ per cent) than in the latter (1 per cent).

(13) The infusion of 1 per cent bicarbonate after a previous infusion of ½ per cent is not productive of beneficial results, the rise in all the pressures being comparatively small. Here, also, the rise in the mean and diastolic pressures is greater than the rise in the systolic pressure would lead one to expect. The subsequent ("late") fall is proportionately much less than is the case in the other secondary experiments.

THE CLINICAL VALUE OF INFUSIONS OF SODIUM BICARBONATE.

Until we know more about the inter-relations of the blood pressures (systolic, mean, diastolic, and pulse pressures) in health and in disease, and until we know more exactly how sodium bicarbonate acts upon the cardio-vascular mechanism, one must be cautious in drawing inferences with regard to the clinical value of infusions of sodium bicarbonate. Nevertheless, the results of the experiments described above indicate that under certain conditions the addition of the bicarbonate to the infused fluid may be expected to have a beneficial action. In extreme cases of that variety of shock which is due to loss of blood, the addition of from ½ to 1 per cent of the bicarbonate to the solution of 0.8 per cent sodium chloride may be of advantage in two respects. In the first place, the rise in all the pressures, but especially in the diastolic pressure, is more pronounced than when the pure chloride is used, and consequently the circulation (so far as the pressures are concerned) can be restored more nearly to the normal condition; secondly, the quantity of fluid required is smaller than is the case with the pure chloride, and hence the greater is the rapidity with

which the solution can be hurried into the circulation, a matter of some importance in desperate cases.

There is, however, one possibility which ought not to be overlooked in the employment of bicarbonate, namely, that of overworking the heart. Some experiments of a series not yet completed show that very strong solutions of sodium carbonate and bicarbonate act as cardiac stimulants to a degree which is simply astounding, and it is probable that such solutions as were used in the above experiments act in part, perhaps chiefly, upon the heart, as suggested by Howell.[15] It would therefore be the duty of the physician to decide in each case whether a cardiac stimulant is or is not contraindicated.

It would seem to the author to be a rational procedure to begin an intravenous infusion with a solution containing bicarbonate, and in this way to hurry the pressures upward, so to speak, and then when the pressures had reached a considerable height the bicarbonate might, if thought advisable, readily be omitted from any subsequent infusion which might be required to maintain the pressure at the desired level.

In conclusion, the author wishes to acknowledge his great indebtedness to his wife, by whom he was assisted in the performance of these experiments.

[15] Loc. cit.

"RED-LEG"—AN INFECTIOUS DISEASE OF FROGS.

By HAVEN EMERSON, M.D., of New York
(Fellow of the Rockefeller Institute for Medical Research),

AND

CHARLES NORRIS, M.D., of New York.
(From the Laboratories of Physiology and Pathology at the College of Physicians and Surgeons of Columbia University.)

Having lost frogs in large numbers every autumn since 1897 from an apparently very virulent epidemic disease, we were led to investigate an unusually severe type of the trouble which destroyed two hundred of a stock of three hundred within two weeks in October, 1903.

In spite of some variations from the morphology as described by previous observers, the identity of the Bacillus hydrophilus fuscus as the essential etiological factor in the disease was definitely determined. In the course of our studies a number of observations were made both upon the pathological conditions found in the frogs, and upon the products of the bacterial cultures, which have not previously been noted. These facts, together with conclusions of practical interest, seemed sufficient reason to offer the following study in confirmation of the work of others, and in the hope that we may give assistance to laboratory workers who have suffered in a similar manner.

Previous Studies.—In an article by F. H. Russell,[7] a very complete picture of the epidemic is to be found, including the results of autopsies and the cultural findings of the Bacillus hydrophilus fuscus. Russell concludes that nothing definite can be stated regarding the mode of infection, but believes the infectious material comes to the laboratory with the frogs and gains access through superficial skin lesions, and lastly, that Chicago water does not contain the infectious agent. In addition to these observations Russell determined the presence of two toxins as

the products of pure culture of the bacillus in bouillon; one having the physiological action of digitalis, the other resembling veratrin in effect.

An organism associated with this disease of frogs was first described as Bacillus ranicida by Ernst,[2] and almost at the same time a bacillus resembling it in many respects was studied by Sanarelli,[8] who found it to be pathogenic for warm-blooded animals as well as for frogs, and named it Bacillus hydrophilus fuscus. He states definitely that his B. hydrophilus fuscus is not identical with the B. ranicida of Ernst. Both observers were led to their studies because of the loss of frogs which they were using in their studies upon anthrax. The bacillus studied by us corresponds more closely to Sanarelli's than to Ernst's.

Ernst made a few inconclusive experiments upon the effect of different temperatures upon frogs inoculated with pure cultures of the bacillus, but is inclined to believe that the frogs are able to withstand the infection better at high temperatures (20° R.) than at low temperatures (7° R.). He also notes that Rana esculenta seems to be more susceptible than Rana temporaria. He lost most of his frogs in spring and summer. Sanarelli tested the portal of entry of the infectious agent by wounding frogs with a clean scalpel and putting some in a tank with sterile water and others in the local tap water. The latter became infected, the former did not. He found the bacillus in the water supply. He concludes that the bacillus is infectious and virulent at temperatures above 30° C.; that it develops quickly in warm-blooded animals; that the filtrate from pure cultures, when injected in quantities of the same volume as were used in causing fatal infections, gave no poisonous effect. He used indefinite doses of "several drops."

Trambusti [10] also was led to his investigations because of losses among frogs in his laboratory. After identifying the bacillus and describing the clinical and pathological findings he studied (1) the physiological effect of pure cultures; (2) the physiological effect of the products of bacterial growth precipitated by alcohol; and (3) the effects of the substances soluble in alcohol.

The effect of (1) was to cause a tetanic condition of muscles and not paralysis. The effect of (2) was similar to the effect of caffein and veratrin upon muscles. The effect of (3) was paralysis.

Roger [5] observed an epidemic in June and merely notes the presence of the bacillus in question and the typical pathological findings. He also confirms Sanarelli's observations upon mammals as well as upon batrachians.

Legrain,[4] in 1888, described a gangrenous septicæmic infection among frogs, but with quite a different appearance and cause.

PRESENT STUDY.—At the time when our last epidemic was at its height, letters were addressed to a number of laboratories in this country and Canada, and, through the courtesy of the professors or their assistants in charge, twenty-one replies were received.* The name often given to the disease in these letters is "red-leg," and this is also the name used by the frog-catchers who state that the disease is well known to them and is seen very generally in the autumn months. The name is useful because it attracts attention at once to the most striking charac-

* We take this opportunity to express our gratitude for the courteous attention given by the following observers to our inquiries, whereby we obtained valuable information:

Professor C. W. Greene,	University of Missouri.
" W. P. Lombard,	" " Michigan.
" Isaac Ott,	
" Colin C. Stewart,	" " Pennsylvania.
" Arthur P. Brubaker,	
" James W. Warren,	Bryn Mawr College, Pa.
" Lafayette B. Mendel,	Yale University.
" W. R. Coe,	
" Wesley Mills,	McGill University.
" Gaylord P. Clark,	University of Syracuse.
" Winfield S. Hall,	Northwestern University Medical School.
" Theobald Smith,	Harvard Medical School.
Dr. W. B. Cannon,	" " "
Professor Graham Lusk,	Univ. and Bellevue Hosp. Medical College.
" George P. Dreyer,	
" Sidney P. Budgett,	Washington University, St. Louis, Mo.
" Theo. Hough,	Massachusetts Institute of Technology.
" George T. Kemp,	University of Illinois.
Dr. Elias P. Lyon,	" " Chicago.

teristic of the diseased frogs, the circulatory congestion of the belly and legs, varying from a faint flush to deep hæmorrhagic injection. The answers may best be arranged under the five questions asked in the circular letter:

I. Have you ever suffered from an epidemic disease among frogs?

Epidemics have been noticed, with characteristic lesions and clinical pictures sufficiently resembling those noted in our epidemic to warrant belief that they were of the same nature in and about Philadelphia and Bryn Mawr, Pa.; Cambridge, Mass.; Ann Arbor, Michigan; Chicago; Baltimore; Brooklyn; Syracuse; Montreal, Canada; Middletown, Conn.; Palo Alto, Cal.; and Columbia, Mo.

A few replies received from observers who had supplies from the same regions as those noted above, especially from Philadelphia and Chicago, state that they have never lost frogs from epidemic disease at any time of year, but this difference in the facts noted by adjacent observers may be accounted for possibly, first by the fact that these observers have had occasion to keep frogs in confinement but a short time, i. e., a week or two, during which time the manifestations of the epidemic are rarely seen. A second explanation seems to be found in the observation of certain precautions by these observers such as care in catching and cleaning and in protecting the frogs when caught from injury or infection or high temperatures.

II. What were the lesions noted, if any?

There was a general agreement as to certain features of the clinical picture and the character of the particular lesions most readily noticed.

Skin lesions, ecchymoses, and ulceration, not merely of the nose from abrasion on the side of the tanks, but of the belly and legs, were noticed by twelve observers. Three others noted ulcers on the feet and nose, with progressive sloughing of the tissues, but none of the characteristics of the epidemic under consideration. Œdematous condition before death among almost all frogs dying with the ulcers and ecchymoses was noticed by five. By two observers, evidences of convulsive muscular action were seen slightly preceding, or at the time of, death.

III. At what time of year have epidemics been seen?

Ten observers state they have seen the epidemics in the warm weather of the autumn, three noting it also in the spring. One noted July and August to be the worst months, most of the others suffering losses chiefly in September and October.

IV. Were any etiological factors noted?

The following are some of the points noted which seemed to different observers to share in causing epidemics: injury during catching, exposure to high temperatures during shipment or in the laboratories, lack of food supply, crowded conditions in the tanks, dirty tanks, tanks with linings of iron, zinc, rough rocks, or with gravel bottoms.

The clinical picture observed in our epidemic was very definite

and uniform throughout the months of October, November, and December; the variations which at first seemed essential differences were later found to be indications of the varying severity of the disease in different individuals or in groups of frogs.

The first thing about the diseased frogs which attracts attention is their sluggishness, which is often so great that they will not move to avoid a hand in the tank. They huddle together instead of jumping away. Chemical and mechanical irritation elicits but slight response. This persists and increases until there is complete failure to respond to stimuli, although if the thorax be opened the heart will be found still beating. On making kymographic tracing with the sciatic-gastrocnemius preparation the curves resemble fatigue curves, in their diminished height, prolonged relaxation period, and increased latent period. This phenomenon was so marked that it was difficult to demonstrate to a student class fatigue by comparison with fresh muscle, so immediately did the muscle fail to respond to stimuli. Distension of the lymph sacs with serum gives them a sodden and bloated appearance, making the sluggishness seem due to weight as well as to paralysis. Dulness of the coat is striking in Rana tigrina and R. viridis, the markings being less distinct, while the colors are paler than in healthy frogs under the same conditions. Congestion of all the ventral surfaces of the body from jaw to tip of hind legs is usual to greater or less degree. The head and trunk are at a much more acute angle with the basis, this position being exaggerated until just before death, when the lower jaw touches the ground and all the legs are sprawling. The attitude is such as to cause the frog to breathe under water for some time before death if there be water only to the depth of half an inch in the tank.

In several instances muscular spasms or tetanic convulsions were noted before death.

The clinical picture given by Russell [7] is similar, but he speaks of muscular spasms before death as appearing frequently, while we noticed them in only about one per cent of all deaths. The results of our autopsies were also similar to those of Russell.[7] Seventy-five frogs of one hundred and fifty which died within

three to five days of their arrival from the marshes of the Delaware and Schuylkill rivers on October 10, 1903, were examined. The lesions were remarkably uniform, but as the epidemic diminished upon the advent of colder weather, we found all grades of severity of lesions, and of suddenness of onset and duration of illness. The skin lesions consisted of hæmorrhagic papules and occasional vesicles, thirty-three per cent of the frogs showing especially large hæmorrhagic exudates under the skin at the joint of the upper and lower extremities, these latter appearing always in the frogs in which other lesions were most severe. This latter class of cases showed no recoveries, death always following rapidly after the appearance of the periarticular hæmorrhages. The extent of the hæmorrhagic skin lesions might well be taken as a measure of the severity of the disease. A few showing diffuse or petechial hæmorrhages into the skin from the mandible to the toes, varying from a slight flush to deep injection, and general hæmorrhages with ulcers, recovered; while a larger number, with a small area of congestion and a few vesicles seemed none the worse for their sickness a couple of weeks later.

The dorsal and ventral lymph spaces were distended with colorless or blood-tinged fluid, according to the severity of the attack, giving the bloated appearance so often noted, and to be explained by the grave changes in the circulating blood. In some of the frogs autopsied within an hour or two after death the heart-blood was found almost colorless and to contain very few red cells.

The tongue was found occasionally flecked with hæmorrhages. Muscular, intermuscular, and periosteal hæmorrhages were often extensive in the hind legs, accompanied by a softening of muscular tissue.

The lungs were the seat of no apparent change, but were in most instances invaded by either the Distomum cylindraceum, or by a small ascarid in large numbers, the former sometimes being encysted in the wall of the lung.*

* This is probably the Rhabdomena (Ascaris) nigrovenosum described by Miss Sheldon[9] in *The Cambridge Natural History*. We have found no other description of a parasite similar to the one seen by us found in the lungs of

When the ascarids were put into a very weak saline solution, they at once became violently contorted and gave out into the fluid countless eggs, through the thin shells of which the embryos could be seen twisting and turning. A still better view of the embryos was had by putting them in glycerine, when the shell previously cloudy though translucent, cleared.

The heart was sometimes pale, but oftener appeared to be the healthiest organ in the animal.

The stomach was uniformly distended with viscid, ropy mucus, and was markedly congested. The intestines were normal except for congestion which was frequently noted, and occasional prolapse of the anal end of the gut. The spleen and liver were soft and mottled.

Specimens taken from the lymph spaces and abdominal cavity of three or four gave uniformly pure cultures.

Essentially the same lesions were found, though in varying degrees of severity in frogs (R. viridis) previously healthy and coming from New Rochelle, N. Y., and (R. tigrina) Chicago, Ill., infected by living in the same tanks with the diseased frogs. The larger frogs succumbed much less readily than the small ones, and the large and vigorous R. tigrina from Chicago proved much more resistant than other varieties under the same conditions, and showed no deaths within a month after their receipt.

In November we secured a lot of healthy R. esculenta and R. tigrina from Ithaca, N. Y., and of these none developed the disease except on inoculation.

During October, November, and December, 1903, our frogs were received as follows:

October—300 from Philadelphia, of which 200 died of the disease. 70 were used before death, and 30 lived without showing any lesions. These consisted chiefly of R. esculenta, among which were a few of the species R. viridis.

November—25 from New Rochelle; a few of these died, but only one with lesions resembling those described above. All were R. viridis.

November—50 from Chicago, of which 10 died, although put in cleaned

---

frogs, but we have not seen the free sexually mature form of the alternating generation, as mentioned in the above description.

tanks, the rest being used for our other studies. All were R. tigrina.

December—50 from Chicago, of which 6 died of the disease, the others being used for inoculation and other studies. All were R. tigrina.

December—45 from Ithaca, N. Y., of which none died except those inoculated with the organisms of the disease. They consisted of R. esculenta and small R. tigrina in about equal numbers.

As the frogs were received they were put in soapstone sinks, each lot separate from the others, and each with an independent supply pipe and exit for water. The city water was used altogether and allowed to trickle from the sink faucet through the galvanized iron wire gauze covering the top of the sink. The sinks were occasionally scrubbed with soap and flushed out with boiling water. The water usually ran cool, but often the overheating of steam pipes raised the temperature to 35° C. None of the frogs was fed at any time.

Inoculation Experiments.—Three sets of inoculation experiments were made, first (A) to test the specific character of the bacillus; second (B), to see if temperature had any effect upon the course of the disease; and third (C), having found very striking effects from cold, to test the extent of the effect of keeping inoculated frogs in cold storage. The results follow.

Series A.—Four frogs (R. tigrina) from Chicago, apparently healthy, were inoculated with one-thirtieth of a twenty-four-hour agar culture of the Bacillus hydrophilus fuscus, obtained in pure culture from an early case of the disease and kept at room temperature. One was killed at the end of twenty-four hours because it presented the typical appearance of œdema, dullness, red-leg, and superficial ulceration. Autopsy showed the usual pathological appearances in the viscera and lymph. Smears of the blood showed advanced degenerative changes in the red cells, and in a few of the cells single and occasionally double bacilli were found; a few bacilli were also found extracellular. Blood cultures showed pure growth of the specific bacterium. Two others which died in forty-eight hours presented the same picture as the one described above, but the blood changes were more marked. The fourth died in fifteen days without œdema, but

with degenerative changes in the viscera and marked blood changes. This animal had thirty-eight ascarids in the lungs. It also showed the specific bacterium in the blood from the spleen but less abundantly than the others.

Series B.—Five frogs (R. esculenta) from Ithaca were inoculated on December 27th with the same strength of emulsion as was used in series A. Two were kept at room temperature and three were put in a cold-storage closet, where the temperature was never over 5° C., and always went down to 0° C. at night. The two at room temperature died within eighteen hours, and on autopsy showed great œdema and visceral degeneration, and the following blood changes: The lymph of the ventral sac was abundant and pink; no red blood flowed anywhere on incision; on opening the heart no red blood appeared; on examining a fresh smear from the heart-blood, diligent search failed to show more than one or two red cells in a field; smears stained with Jenner's stain or fixed in methyl alcohol, and stained with methyl azure and eosin, showed abundant leucocytes and many more stained nuclei of red cells, these being apparently all that were left to represent the red-blood cells.

The other three frogs were kept in the cold until January 16th, and at no time did they show the slightest sign or symptom of disease. They were alive and active on January 30th, having been kept at room temperature since January 16th.

Series C.—Twenty-four frogs were inoculated on December 29th with five minims each of a twenty-hour agar-agar slant culture at 37° C., suspended in 5 cc. of normal saline solution. The injections were all made into the dorsal lymph sac. In other words, approximately one-thirtieth of an agar culture was used. The twenty-four frogs were selected as follows: twelve large frogs of species R. tigrina (class A) from Chicago, among which there had been no disease for two weeks previously. Six small frogs of species R. tigrina (class B) and six of species R. esculenta (class C), both sets from Ithaca, N. Y., among which there had been no disease since their receipt at the laboratory. Four of the twenty-four (two from class A and one from each of the other two classes) were kept at room temperature as controls.

The other twenty were kept in the cold-storage closet under the conditions above described.

At the end of twelve hours the four controls were still alive, though dull and swollen. At the end of twenty-two hours three were dead, one of class A and the two of classes B and C. The other of class A died in twenty-seven hours. Except in the case of the last frog, which showed milder lesions, the findings were those described in the severe cases above cited.

The skin lesions were of the hæmorrhagic and serous character, but there were muscular hæmorrhages also. The blood was almost colorless, and only a few scattered red-blood cells were found. Bacilli were numerous in the lymph and blood. There were two or three of the ascarids and distoma in the lungs of each of these frogs. Of the two removed from the ice-box after a sojourn of four days, both were in perfect health, but they unfortunately escaped from the jars and died, one two days, the other six days, later, apparently as a result of drying upon the floor, as lesions of the disease were absent and no bacilli were found in smears from the lymph and blood.

Of the twenty frogs kept at low temperatures (0°–5° C.), some were allowed to remain continuously in the ice-box, and of these none died at any time.

The others were removed from the ice-box after a sojourn varying from twelve hours to thirteen days, and were then kept at room temperature. The results of this experiment follow:

Those removed from the ice-box after a sojourn of twelve hours died twenty-four hours to three and one-half days later.

Those removed from the ice-box after a sojourn of twenty-four hours died twenty-four hours to three days later.

Those removed from the ice-box after a sojourn of thirty-six hours died thirty-six hours to three and one-half days later.

Those removed from the ice-box after a sojourn of forty-eight hours died two and one-half to four and one-half days later.

Of these the one that died four and one-half days later escaped from the jar and was found dried and shrunken upon the floor, entirely lacking any of the general appearances or lesions seen in the others which died of the disease.

Of those removed from the ice-box after a sojourn of three days, one died seven days later, the other showed no appearance of disease and lived for several weeks.

Of those removed from the ice-box after a sojourn of five days, one died nine days later and one never showed any signs of the disease.

Of those removed from the ice-box after a sojourn of seven days one died nine days later, the other never showed any signs of disease.

Those removed from the ice-box after nine, eleven, and thirteen days showed no signs of disease at any time thereafter.

In other words, of the entire twenty subjected to low temperatures, after inoculation, the nine removed from the ice-box after a sojourn of four days or less showed seven deaths with lesions of the disease, one death without lesions, and one recovery.

The eleven removed from the ice-box after a sojourn of four days or longer showed two deaths, with lesions and bacilli in the blood or lymph, two deaths from accidental exposure and drying, seven recoveries. The last seven showed no signs of disease, and were alive and well thirty-two days after inoculation, although kept at room temperature continuously since removal from the cold. Of those which died, all removed from the cold under three days showed lesions of greater or less severity, and the bacilli were found present in all but one. One frog recovered after a sojourn of only three days in the cold, and the three placed in the cold chamber for only twelve hours lived longer by twelve hours to two weeks than any of those left entirely at room temperature.

The controls (i. e., the four inoculated frogs kept at room temperature) all died, while of the twenty inoculated frogs subjected to low temperatures 50 per cent recovered or died accidentally without the lesions of the disease. Of the deaths among these twenty, 60 per cent occurred in frogs which had been kept in the cold thirty-six hours or less, the proportions of recoveries increasing with the length of time they were kept in the cold.

The water in the jars in which the frogs were kept in the ice-box was always partly frozen during the night, the frogs' heads usually being embedded in the surface film of ice in the morning. The ice melted during the day as the temperature of the chamber rose (i. e., from 0°–5° C.), because of the frequent opening of the doors in daytime.

An observation, which was frequently made in the animal house in which the frogs were usually kept, was that if by acci-

dent or intention the steam heat was allowed to warm the building to 20° C., or if the water running over the frogs in their sink ran warm (30° C.) for a day because of over-heating of adjacent steam-pipes, the spread of the disease was very rapid and fatal among the frogs which had previously shown but occasional cases at intervals of several days.

If the room was warm enough to be healthy for rabbits and guinea-pigs, it was too warm for infected frogs. The nearer the temperatures of the room and water were kept to freezing the healthier were the frogs.

An opportunity was afforded in September and October, 1904, to test on a large scale the efficiency of cold in the treatment of "red-leg." Six hundred frogs of species R. tigrina were received from Chicago on September 1st, and were kept in soapstone sinks at the laboratory temperature. For ten days they appeared healthy, and only occasional deaths from injuries or accidents occurred. Suddenly they began to die in large numbers, as many as 55 being found dead in one day. The gross appearances were identical with those described in previous epidemics, and a bacillus in pure culture isolated from the lymph and the blood proved to be Bacillus hydrophilus fuscus. The average daily loss by death for the week before we made our test was twenty-six frogs.

On September 25th 304 frogs remained, of which number 144 were put in a zinc-lined tray, 2 x 3 feet, in a cold chamber, with temperature varying from 1° C. to 6° C.

On September 26th one death occurred, due to freezing, as the frog was embedded in the ice, but this animal showed absolutely no lesions and inoculations from the blood and the lymph gave sterile plates. Of this lot no others died, the tray being left meanwhile in the ice-box until October 13th; while among the 160 frogs left in soapstone sinks at laboratory temperature, the deaths continued at the rate of ten a day for three days, when 124 of the remaining healthy ones were put in the ice-box. The next day one of these was found dead and showed the typical lesions, but after that there were no further deaths.

During the week following on one occasion the temperature of the cold chamber rose to from 6° C. to 9° C. and remained at

that point for twenty-four hours, and the following morning three frogs in each of the two lots were found with marked lesions. The six were removed to separate vessels, where they lived from one to two weeks. In other words, for the week of September 19th to 25th, when about 500 frogs were kept at room temperature, our loss was 182, and for the week of September 28th to October 5th, when all our frogs (268) were in the ice-box, our loss was one from red-leg, and six already diseased frogs which lived one to two weeks.

Observations on the Blood.—Two further observations were made to control our work: one concerning the presence of the protozoan Drepanidium in the red-blood cells, the other with reference to the anæmia and leucocytosis present in the frogs dying from the disease.

Several of the frogs received in October, 1903, from Philadelphia which were examined showed in stained smears of the blood, in addition to the abundant typical bacillus, a marked leucocytosis and the presence of a hæmatozoan parasite in the red cells. Comparison with the slides and drawings of Dr. Langmann [3] given in his paper on the subject showed the organism to be the Drepanidium he described.

Subsequent search for this organism, to test its association with some of the lesions or characteristics of the disease, showed that it was present in only two per cent of the Philadelphia frogs and in one per cent of the frogs from other sources. There was no reason to consider it in any way related etiologically to the disease in question. This is in accord with the observations of Dr. Langmann, who found this hæmatozoan parasite to be without effect upon the life history of snakes.

In regard to anæmia among the frogs, two distinct causes were found for the severe grade present in a number, one of which may properly be classed as a predisposing factor in some cases, and the other as a direct and constant result of the bacterial infection.

The normal red-cell count in Rana temporaria, according to Rollett [6] and Bethe,[1] is 393,200 per cubic millimetre. In a number of apparently healthy frogs of different kinds the red-

cell count was found by us to vary from 354,000 to 500,000 per cubic millimetre. In a number of frogs with poor musculature and dull skin, but without any lesions of "red-leg" or any leucocytosis, the count varied from 114,000 to 354,000 per cubic millimetre.

In these frogs we found many distomes in the lungs. These parasites live upon the red-blood cells of the frog, which they suck from the pulmonary capillaries. On spreading a distome out upon a slide and compressing it slightly with a cover-glass, the intestine can be seen loaded with the oval nucleated red cells of the frog. Estimating the length of the canal by use of a micrometre scale, following the contortions of the tube, and then counting the number of red cells in a given straight length of the tube, the number of red cells in a single parasite was found to be 300,000. As these parasites were found in some frogs to the number of twenty and over, a total of at least 6,000,000 red cells may be estimated as having been drawn from a frog at a given time.

The total blood bulk of the frogs varied from 0.75 cc. to 1.5 cc. Taking 500,000 red cells per cubic millimetre as a basis of calculation, we have an estimated total number of red cells of 375,000,000 to 750,000,000 per frog, of which 1.6 per cent to 0.8 per cent would be at one time in the digestive canal of the parasites, whose very active digestion makes the process of erythrocytic destruction rapid and progressive.

These parasites were found in healthy frogs, and they were absent in many of the diseased frogs. Thus their presence is not a constant factor, but it seems probable that the anæmia they cause might diminish the resistance to infection or prevent recovery when infection has occurred.

The presence of the ascarid even in numbers up to twenty-six in a single animal appeared to have no relation to any morbid state of the frog. In many instances we found both kinds of parasites in one or both lungs of the same frog, whatever the source of the supply, but the occurrence of the distome was most common in the first supply of Philadelphia frogs which we received.

The constant occurrence of marked and severe blood changes

in the frogs dying of "red-leg" has already been noted, and it needs merely to be repeated that in the severer infections a careful search is required to discover a single red corpuscle in fresh and stained smear preparations of the blood. This observation led us to make a few experiments upon the effects of the filtrate from broth culture grown for a week at 37° C. The filtrate had no effect upon the red corpuscles; but a very different picture was presented by a hanging-drop preparation of a 1-10 saline dilution of normal frog's blood, and an emulsion of a twenty-four-hour agar-agar culture of the bacillus. From being distributed evenly through the drop, the very motile bacilli were, in the course of two hours, seen in clustered masses about the red cells. In three to four hours the cells showed irregular outlines and appeared paler. In ten to twelve hours nothing appeared to represent the cells but the nuclei about which were the now sluggish groups of previously actively motile bacilli. The three facts so often noticed at autopsy, namely, the severe laking of the blood, the presence of numerous isolated red-cell nuclei, and the almost total absence of red corpuscles in the severer cases, find a plausible explanation in the destruction of the red cells, as observed in the hanging-drop preparation.

It must be noted, however, that the great excess of bacilli in proportion to the red-blood cells in the hanging-drop preparation is in marked contrast to the actual conditions observed in the blood of infected frogs. And further, it should be explained that even though the filtrate has no effect *in vitro* upon the red corpuscles of the frog, it must be granted that there is a possibility that the products of the growth of the bacilli in the body may differ from those produced under artificial conditions and tested upon red-blood cells in a test tube.

Morphology and Biology of Bacillus hydrophilus fuscus.—The bacillus isolated from the frogs affected with the so-called "red-leg" has striking biological characters. The following is a description of the morphology, and of the pathogenic and biochemical properties, of the bacillus.

Morphology.—As is the case with many bacilli, the morphology varies considerably, according to the source from which it has

been obtained. The tinctorial properties of the organisms likewise vary, depending upon the stains employed for their demonstration.

Thus in the blood of the frog the bacillus is a fairly thick rod, exhibiting, by Jenner's blood stain, marked bi-polar and other irregularities of staining. A form commonly seen in the fresh blood smears is a shovel-shaped bacillus. The square ends of these forms appear strikingly fringed, and it is by these ends that they often appear in apposition as diplobacilli. With the ordinary dyes, however, the bacterial plasma is uniformly colored. The rods appear single or as diplobacilli, and they are seen within both the white and red corpuscles, in the former being present in considerable numbers, especially in frogs about to die. Just before death, and in the blood of dead frogs, the bacilli may be present in large numbers.

In young cultures on all media shorter and longer rods predominate, while coccoid forms and diplobacilli are also present. The bacillus may be said to be somewhat thicker than the typhoid bacillus, the rods being straight, and on the average the length is five to six times the breadth. In older agar cultures—after four days to several weeks—and on potato the rods remain short and thick, although filaments and forms staining poorly are met with occasionally in abundance. After several days to a week's growth on coagulated ox serum, the organism becomes markedly filamentous and long rods and bizarre involution forms are frequent.

Young cultures stain intensely with all the ordinary dyes. No spores are developed. The bacillus is very actively motile, and does not retain Gram's stain. The thermal death point was not ascertained.

Pathogenicity.—The marked pathogenic action of the cultures on frogs has been described above. On guinea-pigs emulsion of young agar cultures exerts a toxic and rapidly fatal action, one-tenth of a twenty-four-hour culture introduced into the peritoneum or subcutaneously producing death within twelve hours. The site of inoculation in the subcutaneous tissue is the seat of hæmorrhagic infiltration. Guinea-pigs inoculated in the peritoneum

develop a marked peritonitis with blood-tinged exudate. Cultures taken from the heart's blood of guinea-pigs just after death develop a few colonies. The fatal action is thus toxic and septicæmic.

Rabbits, on the contrary, are not susceptible. One-tenth of a 24-hour agar culture produces a slight and transient indisposition, when inoculated by way of the ear vein; and if by way of the skin small abscesses which tend to heal.

The pathogenic action on other animals was not determined.

Cultural Characteristics.—The growth is rapid and abundant upon the usual media, the optimum temperature being about 37° C., although growth is abundant at room temperature and at temperatures as low as 12° C.

The colonies upon agar and gelatin present no diagnostic characters.

The plates on 12 % gelatin, "2 % acid to phenolphthalein," develop colonies which are yellowish by transmitted light, and irregularly marked. When small and young, they are pale and slightly stippled. Liquefaction is complete in two days in the cold room, and well-isolated large colonies cause advanced liquefaction within two days.

The superficial colonies on agar are whitish, somewhat raised and moist, and are coarsely stippled. The circumferential portions of the colony are pale and have even borders, the central portion is decidedly yellowish and is marked by round aggregations. The deep colonies are round or oblong, deep yellow in color, and irregularly lobulated.

Young cultures are odorless. Old cultures, however, acquire a rank odor.

Gelatin Stab.—A small globular-shaped area of liquefaction develops beneath the surface of a gelatin stab culture. The liquefaction increases slowly in extent and is not complete for several weeks. A whitish pellicle is soon formed and a heavy, whitish deposit settles out promptly. No greenish tint was observed. Gelatin stabs grown for two days and then melted remain fluid at room temperature. After three days' growth the gelatin remains fluid when placed in cold storage.

Milk.—Milk is completely clotted in twenty-four hours at 37° C. Liquefaction of the clot is apparent within four days, and is practically complete in ten days. At room temperature clotting and liquefaction occur more slowly than at incubator temperature.

The rapidity and character of these processes vary somewhat in different cultures.

In litmus milk, "1.5 % acid to phenolphthalein," a marked acid reaction promptly develops. The coagulum, instead of being solid, may be finely divided, and then settles out promptly, leaving the fluid clear. The clot never completely disappears, even after four weeks, and may or may not retain its acid color.

In milk rendered alkaline (0.2 cc. $\frac{N}{1}$NaOH to 10 cc. milk) the reaction becomes acid, and a finely divided clot appears in two days at 37° C., which is soon liquefied, the fluid becoming clear. In milk rendered acid (0.2 cc. $\frac{N}{1}$ HCl and 10 cc. milk), the same succession of changes is observed, as also in similar sets of milk-tubes grown at room temperature, the reaction in the latter case taking place more slowly.

The Enzymic Action of Filtrates of Broth Cultures upon Milk and Gelatin.—The sterile filtrates of broth cultures in milk bring about changes similar to those caused by the living bacillus.

The following tests were made:

Litmus milk 10 cc. + 1 cc. broth filtrate (1.3 % acid; culture grown twenty-eight days at 37° C.). Soft clot third day at 37° C., fifth day clot settles out and liquefaction proceeds slowly. After four weeks a small clot sticks to the sides of the tube, the fluid being left perfectly clear.

In litmus milk, to which 0.2 cc. $\frac{N}{1}$ NaOH was added, decolorization begins promptly, coagulation occurs at two days, clarification is complete on the fifth day. The control milk-tube, to which no filtrate had been added, showed the same changes, due to alkaline clarification of the milk, after the fifth day.

In milk rendered strongly acid by addition of 0.2 cc. HCl, clotting and liquefaction of the clot occur as in the milk without addition of acid.

In milk-tubes kept at room temperature clotting is delayed until the tenth day, and the liquefaction is much less extensive and complete than in the tubes kept at 37° C.

The peptonizing action of filtrates on gelatin is rapid and complete.

1 cc. filtrate (growth of four weeks) added to 6 cc. of melted gelatin (12 %) produces permanent liquefaction in two days, 0.5 cc., in five days, while 0.1 cc. requires several weeks to produce slight softening of the gelatin.

The liquefying action of the filtrate (1 cc.), when added on top of a column of gelatin, is much slower, one inch of gelatin being rendered fluid in a week, the action being complete after four to five weeks.

The peptonizing action of the filtrate is greatly accelerated in the incubator.

The filtrates of young broth cultures are apparently more active than those obtained from older cultures.

The filtrate of a three-day broth culture grown at 37° C., reaction 1.75 acid to phenolphthalein gave the following action upon milk:

5 cc. filtrate added to 10 cc. milk, twenty-four hours at 37° C., produced a marked clarification of the milk, in three days the fluid becoming perfectly clear, a small coagulum being left at the bottom of the tube.

1 cc. of the filtrate added to 10 cc. milk causes a solid clot in twenty-four hours, which becomes peptonized in a couple of days. The enzymic action of the filtrate at room temperature is considerably slower than at incubator temperature.

Gelatin, 5–7 cc., to which when fluid 1 cc. of the filtrate is added, remains permanently fluid at room temperature.

Broth.—Marked turbidity occurs within twenty-four hours, and flocculi and a heavy pellicle soon develop.

Potato.—On potato (natural reaction 0.5 % acid to phenolphthalein) at room and incubator temperature the growth is abundant, wet, slightly raised, and yellowish. This yellow color changes in the course of a few days to a reddish hue, assuming later a rusty-colored sputum tint.

In four weeks the potato is covered by a heavy and dull brownish-colored growth, the upper and dry portion of the growth presenting a greenish, metallic lustre.

On the same potatoes rendered alkaline by 0.2 % NaOH, the growth never turns reddish in color, but is abundant, the early citron color turning slowly into a brownish gray.

On acidified potatoes (HCl) the growth is scanty and invisible.

The pigment production on potatoes was found to vary considerably, the reddish tint not appearing constantly, but being much more marked in those potatoes, especially when dry, inoculated from cultures recently isolated. On dry potatoes the

growth was markedly raised and wrinkled, and of an intense reddish brown or terra-cotta color.

The red pigmentation was observed only on the potato.

Slant Agar.—The whitish, moist streak spreads rapidly, and the growth assumes a yellowish tint in the course of time. The yellow pigment production is more marked in the early than in the later subcultures. The yellowish pigmentation of the early cultures became intense when grown for several weeks.

Coagulated Ox Serum.—Abundant whitish growth; no pigment formation; gradual softening of the serum, the liquefaction in the tubes kept at 37° C. being concealed by the evaporation.

Fermentative Action upon Sugars.—When freshly isolated from frogs and tested upon various sugars (Theobald Smith's sugar-free broth with addition of 1 % of sugar), the bacillus fermented mannite, dextrose, and saccharose with the production of acid and gas. The gas production, however, was slight, a large bubble of gas developing in the course of several days at 37° C. Both arms of the fermentation tube became markedly turbid.

Lactose was never fermented with gas production, although the growth in both arms of the fermentation tube was abundant.

After cultivation for a few generations upon agar, the bacillus lost its property of fermenting sugars with gas production. The property of growing in the closed arm of sugar-free broth in Smith's fermentation tube, as shown by the turbidity after twenty-four hours' growth at 37° C., was retained, but in a lesser degree.

The growth in the open arm of the tube containing sugar-free broth and lactose broth is abundant, a heavy, whitish deposit settling out in a few days.

The growth in the open arm of the tubes containing dextrose, mannite, and saccharose broth is not nearly as abundant as in the lactose and sugar-free broth, the cloudiness in both arms being practically similar.

An examination of the following table indicates that the most probable explanation of the slight growth in the open arm is found in the production of acid in the fermentation of the sugars

(dextrose, saccharose, and mannite), which acid inhibits further growth.

TABLE TO SHOW THE AMOUNT OF ACID PRODUCED IN SUGAR-FREE BROTH (1 % ACID TO PHENOLPHTHALEIN), TO WHICH 1 % OF VARIOUS SUGARS HAS BEEN ADDED.

Grown 24 hrs. at 37° C.	*7 days (idem.)*	*4 weeks (idem.)*
Open arm, 1 %	1.1 %*	Not tested.
Closed arm, 1.3 %	2 %	" "
1 % Lactose		
Open arm, 1.1 %	1 %*	Not tested
Closed arm, 1.3 %	2.2 %	2.3 %?
1 % Dextrose		
Open arm, 2.2 %	2.7 %	4.1 %
Closed arm, 2 %	1.8 %	3.2 %
1 % Saccharose		
Open arm, 2.3 %	2.8 %	4.4 %
Closed arm, 1.9 %	2.2 %	3.1 %
1 % Mannite		
Open arm, 2.1 %	2.5 %	Not tested
Closed arm, 1.1 %	2.2 %	" "

Fermentation Reactions in Hiss's Serum Media.—† Hiss's, 1 % dextrose-, mannite-, saccharose-, maltose-, dextrin-and-starch-, litmus serum water (1-3) becomes clotted firmly in twenty-four hours at 37° C.

The clot in the dextrose tubes remains acid and solid for weeks. Softening of the clot begins in the other sugar serum waters after three days, but never becomes liquefied to any extent, except in the dextrine tube. The clot is an acid clot.

In 1 % lactose and 1 % inulin-litmus serum water (1-3), the color, as in the plain sugar-free litmus serum water, remains unchanged, no acid reaction being developed. The serum water becomes opaque, and finally solid in four days. Softening slowly takes place, the clot being almost liquefied in three weeks, and the litmus is decolorized. The clot in these tubes is a "sweet" clot due to a rennet-like ferment.

* Rose tint on adding phenolphthalein.

† Hiss, *Centralb. f. Bakt.*, 1902, xxxi, 302; *Science*, 1902, March 7, p. 367; *Medical News*, New York, 1903, February 14, p. 6.

The filtrates of broth cultures have no action upon the various sugar serum water media, thus 1 cc. of a 28-day filtrate leaves the serum (5 cc.) unchanged after several weeks at 37° C.

From these observations the conclusion seems justified that the fermentation of the sugars is produced through the action of an intracellular bacterial ferment, that is, one which is not extracted or set free, or at least not in sufficient quantities, during the growth of the bacillus in the broth cultures, and is thus absent in the filtrates. In other words, the fermentation of the sugars with acid production is induced only through immediate contact of the living bacillus with the sugar, the filtrates leaving the reaction of the litmus unchanged, as seen in serum water tubes.

In marked contrast to these ferments are those soluble ones we have already described, which peptonize gelatin, and which coagulate and subsequently liquefy or peptonize milk and serum.

We do not know definitely whether the rennet-like ferment and the peptonizing ferment are distinct. The bacillus does not split urea or asparagin with acid production, as 1 % urea and asparagin litmus peptone solutions are unaffected.

Reduction of Nitrates. Indol.—The bacillus rapidly reduces nitrates to nitrites, and indol is slowly produced in Dunham's peptone solution.

An intense color reaction is developed in the nitrate peptone solution in which the bacillus has been grown for twenty-four hours at 37° C., when tested with the sulphanilic or "naphthalymine test." After five days' growth the nitrites are completely reduced, no color reaction being now obtainable.

Growth for twenty-four hours in Dunham's peptone solution develops a marked reddish color on addition of concentrated sulphuric acid, while in tubes grown for two days no color reaction develops. After four days' growth at 37° C. a faint rose tint develops on addition of sulphuric acid and the nitrite solution. The color reaction becomes intense, when the tubes have been grown for from two to four weeks. The deep cherry-red color then obtained varies somewhat from the usual tint obtained with the indol test.

THE IMMUNE BODIES DEVELOPED BY ADAPTATION OF RABBITS TO THE BACILLUS.—Agglutinins and precipitins are developed in rabbits adapted to emulsions of living bacilli, as well as those inoculated with filtrates of broth cultures. Thus in a rabbit inoculated with living organisms an agglutination limit of 1-12,500 in two hours ("microscopical clumps") was obtained, the precipitation limit being slightly above 1-10 (28-day filtrate).

We were unable to demonstrate the presence of anti-ferments in the immune sera in the few tests that were made. It was found that normal as well as immune sera have a marked inhibiting action upon the enzymes of the filtrates, but an accurate study of the differences between the normal and the immune serum was not made. One-tenth of one cubic centimetre of both normal and immune serum prevents the coagulation of 10 cubic centimetres of milk by 1 cubic centimetre of filtrate, and the peptonization of gelatin by 1 cubic centimetre of filtrate.

CONSIDERATION OF SPECIES OF BACILLUS ISOLATED.—We have not succeeded in positively identifying the bacillus. It, however, resembles in most of its cultural characteristics the bacillus described by Sanarelli, the Bacillus hydrophilus fuscus, and also corresponds to Russell's description of this bacillus.

Our bacillus corresponds more closely to Sanarelli's bacillus than to Ernst's Bacillus ranicida. It does not develop pigment on agar, except for a yellowish tint which appears in older cultures in varying intensity, and thus differs from the bacillus as described by Sanarelli and also by Russell. Both of these observers state that their bacillus is pathogenic for rabbits; our organism, however, has only slight pathogenic properties upon these animals.

The variations noted above are, however, insufficient to warrant us in defining a new species and for the following reasons: the bacillus when first isolated by us produced a small amount of gas, whereas later subcultures fermented sugars without gas production. Similarly the pigment production in the early subcultures was more marked than in the later subcultures. The pigment formation, indeed, was so variable that no constant factors controlling its occurrence were determinable.

When first isolated the cultures of 1904 corresponded closely to the initial cultures taken in the fall of 1903, in respect to the pigment formation on potato, the marked enzymic action upon gelatin, milk, and coagulated serum, and the fermentation of sugars and gas production. Unfortunately, we have not been able to determine the loss of gas formation in later cultures of the bacillus of the epidemic of 1904, as was found to be the case in the previous year.

We have not been able as yet to determine, by means of the reaction of agglutination, the group to which our bacillus belongs. A strong anti-serum for Bacillus prodigiosus (1-60,000) fails to agglutinate our bacillus in any dilution, so that we may possibly be justified in excluding it from this group. In determining the group and species characters of a given micro-organism, it is, however, questionable how far cultural features and agglutinability should be taken into account. Thus among the prodigiosus group, races which closely resemble each other in cultural characteristics do not possess similar agglutinative affinities, and an anti-serum developed for one species fails to agglutinate other species which closely resemble the first.

Conclusions.

The epidemics we have observed were due to the presence and growth in the frogs of Bacillus hydrophilus fuscus. This was proved by recovering the bacillus in pure culture from the body fluids of frogs sick or dead of the disease, and the inoculation of healthy frogs with an emulsion of the pure culture, and by obtaining the same clinical picture and pathological findings as in the original diseased frogs; and, finally, by recovering the bacillus in pure culture from frogs inoculated and sick or dying as a result of the inoculation.

The disease is widely distributed throughout North America and Europe, and in this country and Canada is known as "red-leg."

It has been observed by us chiefly in the warm weather of September and October.

The disease is characterized by congestion of the ventral surfaces of the body, with more or less ulceration in, and hæmorrhage beneath, the skin, bloating due to serous exudation into the lymph sacs, gradual failure to respond to stimuli, which symptoms are followed by coma and death, the last being occasionally preceded by tetanic seizures.

After death hæmorrhages into the muscles and degenerative changes in the muscles, spleen, liver, and, to a slight degree, in the intestinal tract, are found. The blood shows an advanced degree of anæmia and leucocytosis.

Predisposing causes of the disease are lesions of the skin, which seem to be the usual portal of entry of the infection, and lowered resistance from heat and from anæmia.

By a series of controlled experiments with inoculated frogs we have shown that, while temperatures a little above freezing have no harmful effect upon the frogs, they completely control all manifestations of the disease in inoculated or diseased frogs, if the frogs are left in the cold for a period as long as seven days; and, further, that even short periods in the cold chamber will bring about a delay of the fatal results in diseased or inoculated frogs.

The anæmia so often found in apparently healthy frogs seems in many cases to be due to the presence in the lungs of the frog of a parasite, the Distomum cylindraceum, which, occurring in sufficiently large numbers in an individual frog, is capable of materially diminishing the available supply of red corpuscles.

Severe laking of the blood, the presence of numerous isolated red-cell nuclei, and great diminution in the number, or almost total absence of the red cells in the diseased frogs, are in proportion to the severity of the infection and due to bacterial action.

The presence of the hæmatozoan parasite, the Drepanidium, does not play any part as a predisposing or exciting cause of the disease.

The ascarid Rhabdomena nigrovenosum, although frequently present as a parasite in the lungs of the frogs, plays no part in causing or promoting the disease.

Precautions to be Observed in Preventing or Checking Epidemics in Laboratories.

If frogs are to be kept for more than a few days, care should be taken to avoid supplies from those marshes where the disease is known to prevail year after year, hence the Delaware and Schuylkill valleys which show the disease in an endemic form had best be avoided.

Frogs should not be caught by barrel-staving or any other violent method which may cause abrasions of the skin. The use of a soft, fine-meshed hand-net is to be preferred to other methods.

Before shipment all injured or very pale or thin frogs, or any showing a suspicion of redness of belly or legs, should be put aside, and the rest washed for twenty-four hours in a full stream of cold water.

Shipment should be made in planed wooden boxes, free from projecting knots, nails, or rough surfaces inside. In warm weather ice in some metal vessel placed in the box is to be recommended for delaying over-heating in transit for short distances.

When received, the frogs should be washed thoroughly in an abundance of clean cold water, and should then be kept in a cellar where the temperature in winter is a little above freezing and in summer about 15° C.

The ideal tank is one with smooth sides and bottom and supplied with fresh cold water. The following description of a tank sent to us by Professor George T. Kemp, of the University of Illinois, is so complete that we take the liberty of quoting from his letter:

"The tank consists of an earthenware sink, 3 x 2 feet and 6 inches deep, lined with a hard glaze. It stands on a frame giving it such a tilt that one-third of the bottom is dry when there is 2 inches of water at the lower end. There is a hole in the lower end fitted with a cork, through which runs a glass tube $\frac{3}{8}$ of an inch in diameter, extending into the sink 2 inches from the bottom. The tank is covered by an ordinary window-sash, in which one pane is replaced by a galvanized wire gauze. Water

trickles into the sink from a pipe opening above the gauze and runs out through the glass tube in the cork in the sink-hole. The frogs can shift from wet to dry as they please. The whole sink can be easily disinfected by using sodium hypochlorite with a scrubbing brush. One hundred frogs can be kept in such a tank."

Treatment of the disease when it occurs may be summed up in the words cleanliness, water, and cold.

If the disease appears among frogs in a tank, those with any lesions, such as ulcerations or hemorrhages, or even faint flushing of the belly or legs, should be put into a separate cleaned tank and kept as near 0° C. as possible.

The rest should be removed from their tank to a clean one after being freely washed with an abundance of cold water; the infected tank should be first scrubbed with soap and water, then with a 1-20 carbolic acid solution, and finally flushed out with fresh water. Prof. Colin C. Stewart, of Dartmouth College, uses inclined tanks in series, one water supply sufficing for all the tanks, the diseased frogs being put in the lowest tank as soon as they have been discovered in any of the upper ones. He finds that this method of weeding out the sick ones, together with one thorough cleansing of all the tanks, will check an epidemic, although those already infected may die.

The use of low temperature will in our experience save almost all of those already diseased.

BIBLIOGRAPHY.

1. Bethe, M.—*Schwalbe; Morph. Arb.*, I, 207–240.
2. Ernst, P.—*Beiträge zur pathologische Anatomie und allgemeine Pathologie*, 1890, viii, 203.
3. Langmann, G.—*N. Y. Medical Journal*, 1899, lxix, 1.
4. Legrain, M. E.—*Revue médicale de l'Est*, 1888, xx, No. 11.
5. Roger, M.—*Comptes rendus de la Société de biologie*, 1893, 709.
6. Rollett, A.—*Stricker; Handb. Lehre von den Geweben*, 1091 and 1141.
7. Russell, F. H.—*Journal Am. Med. Assoc.*, 1898, xxx, 1442.
8. Sanarelli, G.—*Centralblatt für Bakteriologie und Parasitenkunde*, 1891, ix, 193, 222.
9. Sheldon, L.—*Camb. Nat. Hist.*, vol. ii, p. 140.
10. Trambusti—*Beiträge zur pathologische Anatomie und allgemeine Pathologie*, 1893, xiv, 317.

ON THE RATE OF ABSORPTION FROM INTRAMUSCULAR TISSUE.

By S. J. MELTZER and JOHN AUER.

(*From the Rockefeller Institute for Medical Research.*)

Plates I.–III.

The rate of absorption from muscles has never yet been a subject of scientific investigation. In fact, nowhere in physiological literature do we meet the suggestion that absorption from the muscles might differ from that of any other parenchymatous or subcutaneous tissue. Only from the serous cavities, especially the peritoneal, is it generally assumed that absorption takes place more rapidly than from the subcutaneous tissues. In the recent extensive studies of infections and immunity, in which inoculation of animals with virulent, toxic, or antitoxic material is one of the most extensively employed methods of investigation, this is usually accomplished by subcutaneous or intraperitoneal injections, to which injections into the spinal canal, the brain, or the nerve trunks were recently added. In the latter cases, the injections are carried out rather to demonstrate their localized effect than to cause a general systemic infection or intoxication. Intramuscular injection, however, as far as we know, was never yet employed, at least not consciously, as a method of introducing infectious or toxic products into the circulation.

In therapeutics, however, we now and then come across the practice of intramuscular administration of drugs. It originated in the treatment of syphilis with mercury. When at the beginning of the '80's of the last century it was found that hypodermic injections of any compound of mercury gave considerable pain and favored the development of abscesses at the site of injection, the method developed of giving deep-seated injections,

especially in the gluteal muscles where it was claimed that they caused neither pain nor abscesses. It was, however, especially stated by certain authorities[1] "that the absorption from the intramuscular tissue with its fewer lymphatics is much slower than from the subcutaneous tissues where the lymphatics are abundant." For the same reason, namely, of avoiding pain and abscess, the practice of injecting into the gluteal muscles extended to some other drugs, such as digitalis, camphor, etc., which cause pain when injected subcutaneously.

OUR EXPERIMENTS.

We came across the question of the rate of absorption from the intramuscular tissues while studying the effect of suprarenal extract upon the pupil when injected peripheral to a ligature applied to an extremity. In studies of the effect of suprarenal extract upon the paradoxical pupil dilatation,[2] the authors stated that in animals from which one superior cervical ganglion was previously removed, a subcutaneous injection of adrenalin caused a long and lasting dilatation of the pupil on the side without ganglion. The dilatation set in about fifteen minutes after the injection. In order to demonstrate that the suprarenal extract is not oxidized by the tissues, the authors ligated a leg and injected adrenalin peripheral to the ligature. When the ligature was removed after some time, the pupil on the side without ganglion dilated. In this case, however, the dilatation appeared a minute or two after removal of the ligature. The problem which the present writers set out to study was the cause of this rapid onset of the effect of the extract after the use of the ligature. We soon made the observation that in introducing the extract into the part of the extremity peripheral to the ligature, the injections were almost unavoidably made intramuscularly. We then began to study directly the rate of absorption after intramuscular injections, and we reached the remarkable result that, for the substances we have studied so far, the effect of an intra-

[1] Neumann und Finger, *Wiener Medizin. Presse*, 1885, xxvi., 80.

[2] S. J. Meltzer and Clara Meltzer Auer, *American Journal of Physiology*, 1904, xi, 28.

muscular injection stands nearest to that of an injection into the circulation, and is far above that of a subcutaneous application.

The substances we have studied so far are: adrenalin, curare, fluorescein, and morphine. In order to obtain clear results it was desirable to employ substances which give distinct reactions, and at the same time permit a comparison of the different modes of application in the same animal. The most available substance for our purpose was again suprarenal extract, as its absorption into the blood is capable of giving us three distinct reactions: the rise of blood-pressure, the dilatation of the pupil, and the characteristic prostration. The effect upon the blood-pressure has, as a method of investigation, an advantage over the other reactions mentioned in that as it permits a graphic presentation of the results.

THE EFFECT OF INTRAMUSCULAR INJECTIONS OF ADRENALIN UPON THE BLOOD-PRESSURE.

The blood-pressure experiments, as well as all other experiments recorded in this paper, were made exclusively on rabbits.

In the experiments on blood-pressure the carotid artery was connected with a mercury manometer, by means of which the variations in the pressure were recorded on a slowly revolving smoked drum. During the operations the animals were anæsthetized with ether; during the further course of the experiments, in some cases the animals were kept under mild anæsthesia by ether, or a small dose of morphine was injected. In a few cases curare was given intramuscularly.

It is worth while mentioning that, in studying the effect of suprarenal extract upon the blood-pressure in rabbits, we were greatly hampered by the favoring effect of the extract upon blood-clotting.[3] As soon as the extract was absorbed into the circulation we could hardly obtain two consecutive tracings without being compelled to free the cannula and connecting tubing from clots. In some cases a clot ended the tracing before the entire effect of the extract was over. In other cases the

[3] Vosburgh and Richards, *American Journal of Physiology*, 1903, xi, 30.

cleaning of the tube was of no avail, as the clot was formed far down in the carotid artery.

On the other hand, a *saturated* solution of magnesium sulphate, with which cannula and connecting tube were filled, proved to be a source of great danger to the animal. When, after a struggle, the pressure sank for a few seconds, the solution of magnesium sulphate rushed backward through the central end of the carotid and reached the aorta, the animal was liable to die within a few seconds without any asphyxia and with practically no struggle. We preferred using less concentrated solutions of magnesium sulphate, and contending with the difficulties of clotting rather than risk the effect upon the circulation. We lost a number of animals in rapid succession, due to this effect of the magnesium salt. We bring out this point here because we believe that some physiological experimenters are not fully familiar with the eminently toxic effect of magnesium sulphate when brought directly into the circulation.

In studying the effects of intramuscular injections of suprarenal extract upon the circulation, we had to compare them with the effects of other modes of administration, i. e., with the effects of subcutaneous, intraperitoneal, and intravenous injections of the extract. This comparison extended to the questions of the time of the onset, of the effect after injection, the character of the development, and the degree of the increase of the pressure which is caused by each mode of injection. For various reasons we had to neglect in this study the question as to the duration of the effect due to each mode of injection.

Of the modes of administration of the extract, we have compared the intramuscular most extensively with the subcutaneous injections from which they are generally but little distinguished. We made but few comparisons with intravenous injections, considering this comparison of no particular importance in the present study; and we also made but few comparisons with intraperitoneal injections, for reasons which we shall state later. Of the results which were obtained, the greatest value had to be attached to those which were derived from comparisons made on one and the same animal, as in comparisons between the

results obtained from different individuals the element of individuality might come into play. But in the comparisons on the same animal the question of sequence had to be taken into consideration, since a preceding injection always has a better effect than the following one.[4] We have therefore, in some experiments, varied the sequence of injections, i. e., in some experiments a subcutaneous injection was given first and this was followed by an intramuscular one; and in others we reversed the order. However, since a preceding intramuscular injection impairs incomparably more the effect of a subsequent subcutaneous injection than a subcutaneous could effect a following intramuscular one, we began the majority of experiments with a subcutaneous injection.

Between two injections we usually permitted intervals of fifteen to thirty minutes to elapse, sometimes letting the drum run continuously or obtaining short curves every two minutes. Although in most of the experiments we waited with the second

4 It has been stated by many observers that in consecutive intravenous injections of suprarenal extract the effect upon the blood-pressure is gradually diminished. This gradual decrease in the effect was looked upon as a sort of process of adaptation or immunization. In previous unpublished experiments with subcutaneous injections of the extract we have noticed that by this mode of administration we rarely obtained an effect from a second and certainly not from a third injection, although they were given each time in another place and the effects of subsequent intravenous injections were in no way impaired. The above given explanation of adaptation, etc., will not hold good in this case. In our present studies we also found that in repeated intramuscular and peritoneal injections of adrenalin the effect upon the blood-pressure is rapidly decreased. Our interpretation of this phenomenon is as follows: In a previous paper (Meltzer and Auer, *Transactions of the Association of American Physicians*, 1904, xix, 207) we have demonstrated that the introduction of suprarenal extract into the circulation retards the absorption from the tissues into the blood, and also the transudation from the blood into the tissues. When, therefore, a subcutaneous, an intraperitoneal, or an intramuscular injection of suprarenal extract once produces an effect upon the blood-pressure, it has then of course reached the circulation and causes therefore a retardation of the further absorption of the extract from the tissues; hence the smaller effect of a second injection. Possibly the same explanation might also hold good for the gradual reduction of the effect of repeated intravenous injections of the extract, since transudation also becomes retarded, and, since even in intravenous injections, the vascular intima has to be permeated before the extract can exert its influence upon the muscle fibres of the media.

injection until the blood-pressure reached its original level or fell below it, we cannot claim that even in those cases the effect of the preceding injections had actually passed off, since the frequent cleanings of the cannula and the necessity of letting blood escape in order to drive out a deep-seated clot, might have interfered with the absolute pressure. For this reason we refrained, as stated above, from utilizing the data regarding the duration of the effects of these injections.

We did not attempt to make an exhaustive study of the effects of subcutaneous and intraperitoneal injections of the suprarenal extract upon the blood-pressure, since the chief purpose of our present investigation was a study of intramuscular absorption, and not of the effect of suprarenal extract upon blood-pressure. However, we have in the present and in previous studies acquired a few data regarding the effects upon blood-pressure of subcutaneous, as well as of intraperitoneal, injections of suprarenal extract which we are about to report briefly.

SUBCUTANEOUS INJECTIONS.

In the physiological and the pharmacological literature upon suprarenal extract we frequently find the brief statement that subcutaneous injections of the extract exert no influence upon blood-pressure. In the medical literature, however, some scattered remarks are met that subcutaneous injections do increase the blood-pressure. We are not aware that any definite data on the subject accompanied by tracings or figures have been published anywhere. From our present studies we know that if special care is not taken, the hypodermic needle will sometimes get under the fascia of muscles and the result will be a definite effect upon the blood-pressure, but it is then not from a subcutaneous, but from an intramuscular injection of the extract. In the light of our present experience we have reason to distrust therefore not only the statements of others, but also our own previous observations when made while we had no idea of the necessity of sharply distinguishing between subcutaneous and intramuscular injections. However, as will presently be shown,

there is something characteristic about the blood-pressure curve obtained from a subcutaneous injection which assists in identifying it.

From our experiments we may state in a general way that subcutaneous injections of suprarenal extract in rabbits will undoubtedly influence the blood-pressure, but the degree of the effect is in no way comparable to that obtained by intravenous injections. Furthermore, the effect is very variable; while sometimes even a comparatively small dose will bring out a relatively powerful effect, a large dose cannot always be relied upon to cause a noteworthy rise of pressure. As a rule, a dose of 1.5 cc. of commercial adrenalin (1 :1000) will cause the characteristic prostration of the animal and the ears will show an absolute blanching, while at the same time the general blood-pressure may show a gradual rise not exceeding perhaps ten millimetres of mercury. On the other hand, doses of 0.4 or 0.5 cc. of adrenalin per kilo animal will, as a rule, exert no influence whatsoever upon the blood-pressure of the rabbit. (See Figs. 4, and 6.) But in some exceptional cases even such a small dose might unexpectedly produce a comparatively powerful influence. Figure 1, presents such a powerful effect of a first subcutaneous injection of 0.8 cc. adrenalin in a rabbit weighing 2200 grams. In a series of experiments on more than thirty rabbits such effects were obtained only twice.

Figure 1 might serve also as prototype for the character of the normal curves, which are obtained after a subcutaneous injection, no matter whether the effect be small or large. While the rise of pressure after intravenous injections is characterized by a very short latent period, steep rise and gradual descent, the entire effect lasting only from four to six minutes, we see in subcutaneous injections the effect only beginning minutes after the injection, the pressure rising slowly, remaining at the maximum for some time, and then gradually descending to the original level, the entire course frequently extending over a period of twenty to thirty minutes.

The original height of the blood-pressure seems to be an influential factor upon the effect of a subcutaneous injection. When

the blood-pressure is low we may reasonably expect a noteworthy rise after such an injection. However, a rise of more than 25 to 30 millimetres of mercury is under all conditions rather an exception.

We have already stated above that, as a rule, only the first injection is distinctly effective; the effect of a second injection, if there is any, is already perceptibly reduced, and there is rarely any effect after a third injection. However, after an interval of a few hours an injection might again become effective.

A struggle of the animal seems to have a tendency to bring out the effect of a subcutaneous injection. In normal conditions, struggle, as a rule, causes a fall of short duration, and if there is sometimes a moderate rise of pressure, it returns after a few seconds to the previous level. After a subcutaneous injection of an ineffectual dose of adrenalin we noticed, however, in a few instances that a struggle was followed by a sudden moderate but long-lasting rise of pressure (see Fig. 10.) Possibly struggle is instrumental in causing rapid absorption of some of the injected adrenalin.

INTRAPERITONEAL INJECTIONS.

Our experience with the effects of intraperitoneal injections of suprarenal extract were a surprise and a puzzle to us. In the majority of the experiments we have seen either no effect or a rise of only 10 or 20 millimetres. (See Figs. 13 and 14.) We met only with two exceptions; but in these two instances the rise which followed immediately after the injections was so powerful and so very like the effect of an intravenous injection, as to raise the doubt whether the injections were not indeed made intravenously. At first we made the injections by means of a hypodermic syringe through the intact abdominal wall. We soon discarded this method and carried on the injections either through a small nick in the abdominal wall or through wide incisions, trying at the same time to carry the adrenalin to different parts of the peritoneum, with the above stated results. Of the two exceptions mentioned, one was obtained by plunging a hypodermic syringe through the intact wall,

the other by pushing a hard catheter through a small abdominal incision, between liver and diaphragm. In the first instance the needle might have entered a blood-vessel, and in the second the catheter might have injured the surface of the liver, thus facilitating the entrance of the extract directly into the circulation.

How are these results to be explained? Can it indeed be a fact that suprarenal extract enters but little into the circulation when applied directly to the peritoneum, possibly on account of its intense local action on the blood-vessels of the peritoneum, thus preventing its absorption into the blood? We intend to take up this question again in an investigation specially devoted to it, and shall therefore refrain from discussing it here any further.

INTRAMUSCULAR INJECTIONS.

Our experience with intramuscular injections of suprarenal extract has been more extensive and more varied than with the other methods. The results, however, were so precise and uniform that they can be stated briefly. The effect of an intramuscular injection of adrenalin upon the blood-pressure approaches in every respect that caused by an intravenous injection. The rise sets in a few seconds after the injection and reaches within a fraction of a minute its maximum, which can be considerable. A dose of 0.3 or 0.4 cc. of adrenalin may produce a rise of 40 to 50 millimetres of mercury. Figures 5, 9, 15, will illustrate these effects better than they can be described by words. These curves were obtained from intramuscular injections of such small doses of adrenalin that if applied subcutaneously they exerted an insignificant influence or none at all upon the blood-pressure. The effect of an intramuscular injection differed from that of an intravenous only in degree; the rise of pressure was not as large and not entirely as sudden as after intravenous injections. Furthermore, an intravenous injection still responded with a fairly good rise when, after repeated intramuscular injections, no reaction could any longer be obtained. We have, however, to state that in intramuscular injections the response was not exhausted until three or more consecutive

injections had been made, although each consecutive reaction became distinctly smaller.

A feature of our intramuscular curves is the frequent appearance of so-called "vagus-pulse," i. e., the heart-beats become larger and often less frequent, as was ascribed by Biedl and Reiner [4] (in tracings from intravenous injections) to a stimulation of the vagus centre in the medulla. From a comparison of the blood-pressure tracings in the experiments of many writers on intravenous injections with the tracings obtained by us after intramuscular injections, it would appear to us that the vagus-pulse is a much more frequent phenomenon after an intramuscular administration of suprarenal extract than after an intravenous one. We record here our observations without further comment as to the possible nature of this difference. Sometimes the vagus-pulse sets in before the pressure has reached its maximum; then the pressure will rise still higher after the cessation of the vagus-pulse (see Figs. 5 and 14); or the large pulsation sets in after the rise has attained its maximum, and it will then be accompanied by a slight fall of pressure (see Fig. 15), or there will be large vagus-pulses throughout the entire effect following the injection, the rise of pressure in this case not being very high (see Fig. 7).

In some instances it seemed that also after an intramuscular injection a struggle would increase the rise of blood-pressure, or cause a second rise after there was already a tendency to drop.

As to the duration of the rise we cannot, for reasons mentioned, make any positive statements. We may, however, remark that the general impression received from a survey of our tracings is that the effect upon the blood-pressure lasts somewhat longer after intramuscular injections than after intravenous ones.

In the majority of the experiments the injections were made into the muscles of the back, in the lumbar region, and we had not a single failure. We have, however, established that the effect is the same if the injections are made in any other muscle.[5]

[4] *Pflüger's Archiv*, 1898, lxxviii., 385.

[5] Among the tracings in our possession from previous studies on the effects

In injecting into other muscles of the rabbit's body care has to be taken that the needle remains within the muscle, as with the thin muscles of the animal it might easily happen that the needle enters the loose areolar tissue between groups of muscles, in which case the effect will be the same as from the subcutaneous tissue.

EXPERIMENTS WITH THE PARADOXICAL PUPIL REACTION.

As was already stated above, S. J. Meltzer and Clara Meltzer Auer [6] have found that some time after removal of the superior cervical ganglion (twenty-four hours in rabbits and forty-eight hours in cats) a subcutaneous injection of adrenalin caused a dilatation of the pupil on the side without the ganglion, which would last for hours. The doses of adrenalin employed were about 1.2 to 1.5 cc. for a "large rabbit," and the reaction appeared "a few minutes" after the injection. In some instances even such a small dose as 0.6 cc. caused some dilatation. The authors had at that time, of course, no reason for distinguishing between subcutaneous and intramuscular injections.

We now resected the superior cervical ganglion in a number of animals and studied the effect of the extract according to the tissue injected, with the following results:

When a dose of adrenalin of 0.4 cc. per kilogram of animal was injected intramuscularly, the pupil on the operated side began to dilate within a fraction of a minute and reached the maximum within two minutes. Such a small dose has nearly no effect upon the pupil in subcutaneous application. When a large dose was injected subcutaneously, the pupil on the operated side dilated ten to fifteen minutes after the injection. When the pupil once became dilated *ad maximum*, the dilatation lasted apparently just as long after intramuscular injection as after an effective subcutaneous one. Doses smaller than 0.4 cc. per kilo

of subcutaneous injections of suprarenal extract there was one from a "subcutaneous" injection of a small dose of adrenalin made into the pectoral muscle (Fig. 2). It looked exactly like the following tracing (Fig. 3), which was obtained with a still smaller dose from an intravenous injection. We know now that it was not due to a subcutaneous but to an intramuscular injection.

[6] S. J. Meltzer and Clara Meltzer Auer, *Amer. Journ. of Phys.*, 1904, xi, 28.

given intramuscularly would often cause only a moderate dilatation of the pupil, which would set in later and disappear sooner. In no case have we observed that an effective intramuscular injection has ever caused any dilatation of the pupil on the normal side. This fact distinguishes the intramuscular application from the intravenous, after which even the pupil on the normal side becomes dilated for a fraction of a minute.

THE EFFECT OF THE SUPRARENAL EXTRACT UPON THE SKELETAL MUSCLES.

The early writers on the influence of suprarenal extract have noticed its general prostrating effect upon the animal. Oliver and Schaefer [7] have observed that ten minutes after a subcutaneous injection of the extract into a frog "its movements have usually become languid," and after another 5 minutes" its voluntary power over the muscle is greatly diminished." In the rabbit, "half-an-hour after a large subcutaneous dose, the animal may become listless, and may, when interfered with, move languidly." In our experience a dose of about 2 cc. of adrenalin, when given to a medium-sized rabbit subcutaneously, would produce a distinctly prostrating effect. The animal would lie on its belly, all four extremities stretched out, the chin resting on the table and the head turned sideways by its own weight, and would move only with great effort. This effect appeared fifteen to twenty minutes after the injection. After intramuscular administration, however, the prostrating effect already appeared a minute or two after the injection of 0.6 or 0.8 cc. per kilo, a dose which exerts hardly any effect in subcutaneous injections. We have to add that doses larger than 0.8 cc. can be fatal to the rabbit when given intramuscularly; the animal may die even within a few minutes after the injection, while we have never met with such accidents in the practice of subcutaneous injections even after larger doses.

EXPERIMENTS WITH CURARE.

The experiments with curare were very instructive. They brought out the difference between subcutaneous and intramus-

7 Oliver and Schaefer, *Journal of Physiology*, 1895, xviii, 227.

cular injections in a striking manner. We succeeded in finding the doses which in subcutaneous injections brought out no perceptible effect, while after intramuscular injection all the voluntary muscles were paralyzed, at least all except the respiratory muscles. We shall illustrate with a few protocols.

Experiment I. Nov. 15. 1904.—1. Gray male rabbit, 1300 grams injected subcutaneously with 0.25 cc. of a 1 % solution of curare (0.2 cc. per kilo). Rabbit quiet at first. Practically no effect.

2. White and gray male rabbit, 1560, injected intramuscularly, (right thigh) 0.3 cc. of above solution of curare (0.2 cc. per kilo). (The same solution was used in all experiments.) After seven minutes the paralysis developed to such degree that artificial respiration was necessary. Animal used for a blood-pressure experiment.

Experiment II. Nov. 16.—1. Rabbit number 1 of Experiment I was again given subcutaneously 0.25 cc. of curare (0.2 cc. per kilo). Practically no effect.

2. Black and white male rabbit, 1320, received intramuscularly in right thigh, 0.25 cc. curare (0.2 cc. per kilo); paralyzed; artificial respiration necessary. Used for a blood-pressure experiment.

Experiment III. Nov. 17.—Rabbit number 1 of the two previous experiments, during which it had twice received subcutaneously 0.2 cc. curare per kilo with no effect, was now given intramuscularly (thigh) 0.25 cc. of curare (0.2 cc. per kilo). After six minutes practically paralyzed.

Experiment IV. Nov. 30.—1. Brown female rabbit, 1540, injected intramuscularly (back) with 0.29 cc. of the 1 % solution of curare (0.19 cc. per kilo). After five minutes animal unable to move, all voluntary muscles profoundly affected except the muscles of the respiratory mechanism. Two hours later still paretic, but able to move again.

2. Brown female rabbit, 1435, received subcutaneously (back) 0.27 cc. of the solution of curare (0.19 cc. per kilo). Watched for two hours; no effect whatsoever.

The effect of an intramuscular injection of curare resembles

greatly that of an intravenous one. The effect sets in quite rapidly and lasts for some time, perhaps even longer than after an intravenous injection. We have employed doses so small as would hardly be sufficient to ensure absolute rest even in intravenous injections. When at the end of a blood-pressure experiment, for which some of the rabbits with intramuscular injections of curare were utilized, the animal was permitted to die, the asphyxia would bring out perfunctory convulsions. Even during the experiment one or the other animal would show once in a while a faint struggle, especially when the dose was only 0.18 cc. per kilo; and we have never added any more curare during the experiment to ensure absolute rest, as is frequently done in intravenous injections. We believe that the intramuscular injections of curare will prove to be a practical method in experimentation on animals, as it is much more convenient and less dangerous than intravenous injection.

EXPERIMENTS WITH FLUORESCEIN.

The effects of the subcutaneous injection of fluorescein we have extensively described in our experiments upon the influence of suprarenal extract upon absorption and transudation. The skin and mucous membranes become gradually more or less distinctly yellow. Following larger doses, Ehrlich's line in the pupil appears after some time. By intravenous injection the animal is stained intensely yellow, almost in an instant. We have now tried an intramuscular injection of fluorescein, comparing it with the effect of a subcutaneous administration of a similar dose. We shall quote a protocol of such an experiment.

Nov. 16.—White and gray rabbit, 1050 grams received subcutaneously (back) at 4.05 P.M., 2 cc. of a 5 % solution of fluorescein. At 4.09 2.5 cc. of the same solution of fluorescein was injected intramuscularly (back) into a gray rabbit, 1300 grams (both animals received 1.5 cc. per kilo). At 4.12 conjunctivæ of both animals show yellow tint, but much more intense in the "intramuscular." 4.13, "intramuscular" shows yellow nose, "subcutaneous" still pink. 4.20, "intramuscular" much more

yellow in all parts than "subcutaneous." Ehrlich's line appears at 4.20 in the left, and at 4.25 in the right eye of the "intramuscular"; no Ehrlich line in either eye of the "subcutaneous." 4.29, both pupils of "intramuscular" perfectly green; the pupils of "subcutaneous" black, no tint of green.

Fluorescein is absorbed incomparably more rapidly and in larger doses from the intramuscular than from the subcutaneous tissue. However, it should also be noted that the effect of an intramuscular injection of fluorescein cannot be compared in rapidity with that of an intravenous injection of the same dose and strength.

EXPERIMENTS WITH MORPHINE.

We have finally to record the results of our experiments made with morphine. They are fully in accord with the results obtained with the injection of the other substances recounted in the preceding pages. The symptoms brought on by morphine are inappropriate to show the differences in such a characteristic manner as was observed in the experiments with the other substances, especially the onset and the development of the effects are too slow to permit sharp comparisons. However, when the experiments are made simultaneously on a series of animals, the differences of the two groups are unmistakable and are perceived at a glance. The differences between the effects of intramuscular and subcutaneous injections of morphine presented themselves pronouncedly when both kinds of injections were practised on the same animals, of course after proper intervals. The following protocol will illustrate our statement:

Nov. 11.—Four rabbits received at about the same time 8 milligrams of morphine sulphate per kilo of animal. A and B received it intramuscularly; C and D subcutaneously. Within a few minutes after injection, the difference between the two groups was marked: A and B lying on their bellies, legs stretched out, heads resting on the table, and ears flat on back. C and D quiet, but head and ears erect and body not stretched out. Thirty or forty minutes later the difference in the behavior of the two groups still marked; the respirations, too, are much

slower in the "intramuscular" group. An hour after the injections the difference between the two groups now less marked. Next day animals normal.

Nov. 15.—The same four animals again received 8 milligrams of morphine sulphate per kilo. But now the order was reversed: A and B were injected subcutaneously, C and D intramuscularly. Now C and D after eight minutes became droopy, lying on belly, etc., while A and B (now subcutaneous) show little or no effect. After twenty-four minutes all four animals were put on the floor: A and B hopped off to a corner, C and D remained on the spot where they had been put, in a droopy, somnolent condition. The difference in the behavior of the two groups was well marked, and the contrast in the behavior of each group in both experiments was indeed striking.

By the foregoing experiments we believe to have firmly established, at least for the substances we have experimented with, that absorption from intramuscular tissue is incomparably faster than from subcutaneous tissue. The effect of an intramuscular injection stands in value very near that of a direct injection into the circulation. That these injections into the muscles were not indeed intravenous injections, i. e., that, accidentally, each time the injection was made into a blood-vessel, needs hardly a refutation; the constancy of the results speaks sufficiently against their accidental nature. It could, however, be assumed that, the muscles being provided with many blood-vessels, an intramuscular injection is unavoidably an injection into the blood-vessels. Hence the rapid effect of an injection into the muscles was actually only the effect of an intravenous injection, and does not signify that a rapid absorption from the muscles into the blood-vessels takes place normally; in other words, our experiments do not prove that in the normal metabolic processes within the muscles the rate of absorption is different from that of the subcutaneous tissue. But the following facts speak against such an assumption. The rise of blood-pressure which was caused by an intramuscular injection of suprarenal extract was rarely as great as that which is caused

by an intravenous injection of a similar dose. Furthermore, after repeated intramuscular injections a further injection by the same method would have no effect, while subsequent intravenous injections would still continue to cause a considerable rise of pressure. In the effect upon the pupil there is a distinct difference between the effects of intravenous and intramuscular injections. An intravenous injection of suprarenal extract would always cause a dilatation of the pupil, although of short duration, even when the superior cervical ganglion was intact, while we have not observed that an intramuscular injection ever caused any dilatation of the pupil on the unoperated side of the animal. Finally, the most striking differences between intramuscular and intravenous injections have been observed in our experiments with fluorescein. After intravenous injections, even with much smaller doses than those which we have employed in the present experiments, the effect is instantaneous: the conjunctivæ become orange-yellow, the pupils deep green, etc. within a few seconds after the injection. While with intramuscular injections the pupils did not become green until nearly half an hour after the injection, and the entire animal was by far not so deeply colored as after an intravenous injection.[8]

We therefore entertain the view that in intramuscular injections the fluid does not enter directly into the blood, but is at first deposited between the muscle fibres and is carried thence into the blood by some process of rapid absorption.

We have to add that, in our opinion, the difference between the absorption from intramuscular and subcutaneous tissues is only a matter of degree. We have seen in our previous experiments with fluorescein that one minute after a subcutaneous injection there was already fluorescein present in the serum of the blood. The difference consists only in the quantity which becomes absorbed in a unit of time, and the quantity absorbed is apparently so much larger from the intramuscular tissue than from the subcutis that it produces strikingly different results. Whether some substances are absorbed better from the intramuscular tissue than others, we cannot state for the present.

[8] S. J. Meltzer and John Auer, l. c.

The fact that the difference between the effects of intramuscular and intravenous injection is much greater with fluorescein than with, for instance, curare, would speak for such a possibility. Future researches in this direction will throw light on this problem, as well as on many others which are bound to present themselves on the basis of our present investigation.

Why is the absorption from the muscles so much more favorable than from the subcutaneous tissue? Possibly because the unit of space in the muscles contains more blood and more blood-vessels, and is therefore capable of taking up more liquid injected into it in the same unit of time. Possibly the readiness of the blood-vessels of the muscles to respond with vaso-dilatation to local and reflex stimulation is also a factor in this process. It is possible also that the capillary walls of the muscular tissue are much more permeable than the capillary walls of the subcutaneous tissue. We shall, however, for the present not commit ourselves to any theory or enter even into a discussion of the merits of the factors concerned, as this would be premature. Not only has a new fact been observed, but a new field has been opened, which is bound to yield many more new facts capable of throwing more and better light upon the underlying causes. The application of the new point of view may be manifold. In physiology, for instance, we ought to learn in general, from the observations communicated in the preceding pages, that absorption from the tissues of the body is not a single, uniform process, but that the rate and the mode of absorption may vary with each tissue and organ. In particular we might study and come to understand the rapid interchange between the metabolic products of the muscles and the general circulation, the rapid effect which the catabolic products of the active muscles exert upon the mechanisms of circulation and respiration, and many other problems. In pathology we might study and come to understand whether and why infections and intoxications from the muscles occur less readily than from subcutaneous tissue, and why œdema of the muscles is a much less frequent phenomenon than œdema of the subcutaneous tissues. In the experimental studies of infection and immunity, the intramuscular incorporation of virus, toxin,

and antitoxin should present a distinct method of study, and the results should be compared with those of other methods in vogue. In pharmacology the method of intramuscular injection should receive special attention. The action of pharmacological products which are effective in small doses should be tested by intramuscular injection with regard to the rapidity of action as well as the duration of the effect. The permissible dosage should be established. We have seen above that a dose of curare, for instance, which was absolutely harmless in subcutaneous application, was nearly fatal when given intramuscularly. In therapeutics, intramuscular injection might offer the advantages of an intravenous administration without its dangers. On the other hand, a combination of intramuscular and subcutaneous injections might ensure rapidity and intensity together with long duration of action.

EXPLANATION OF FIGURES.

The line on each figure represents variations in blood-pressure written by the mercury manometer. Base lines and time markings were omitted in printing the curves. The tracings were obtained from the carotid artery of rabbits after subcutaneous, intraperitoneal, and intramuscular injections of commercial adrenalin chloride (1:1000).

The first three figures were obtained in previous studies on the influence of adrenalin on the blood-pressure, administered by subcutaneous injection. (The time was marked in five seconds.)

PLATE I.

Fig. 1 represents a part of such a tracing; twenty-eight minutes passed before the elevation reached the original level. Only two curves with such a rise were obtained in an extended series of experiments.

Figs. 2 and 3 were obtained consecutively on the same animal. Fig. 3 was obtained by intravenous injection of 0.3 cc. Fig. 2 was obtained by an injection of 0.4 cc. in the region of the left pectoral muscle. As the autopsy revealed no injury to a blood-vessel, it was then taken for an exceptional effect of a "subcutaneous" injection. It was, however, apparently the effect of an intramuscular injection.

A cross on the curves marks the end of an injection.

Fig. 4.—1375 grams. 1 cc. subcutaneously, upper abdomen. *First* injection.

Fig 5.—Same animal. *Second* injection; 0.8 cc. intramuscularly, left back, lumbar region.

PLATE II.

Fig. 6.—2500 grams. *First* injection; 1 cc. subcutaneously, left upper abdomen.

Fig. 7.—Same animal. *Second* injection; 0.8 cc. intramuscularly, left back, lumbar region.

Fig. 8.—1500 grams. *First* injection; 0.6 cc. subcutaneously, abdomen.

Fig. 9.—Same animal. *Second* injection; 0.6 cc. intramuscularly, left back, lumbar muscles.

Fig. 10.—Same animal. *Third* injection; 0.6 cc. subcutaneously, right upper abdomen.

(Note that Figs. 8, 9, and 10 are consecutive.)

PLATE III.

Fig. 11.—1320 grams. Curare; artificial respiration; 0.8 cc. intraperitoneally through catheter in abdominal wall. *First* injection.

Fig. 12.—Same animal. *Second* injection; 0.8 cc. intramuscularly, left back, lumbar region.

Fig. 13.—2045 grams. *First* injection; 0.8 cc. intraperitoneally through cut in abdominal wall.

Fig. 14.—Same animal. *Second* injection; 0.8 cc. intramuscularly, left back, lumbar region.

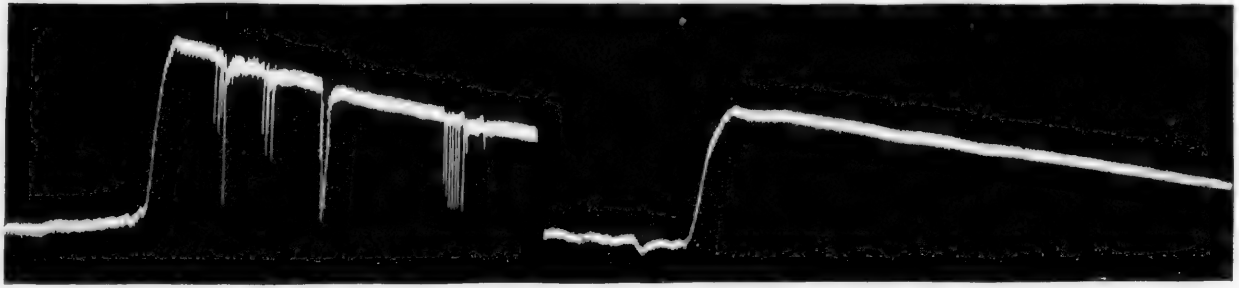
Fig. 15.—1540 grams. Curare; artificial respiration; 1 cc. intramuscularly, left back, lumbar region. *First* injection.

Fig. 16.—Same animal. *Second* injection; 1 cc. intraperitoneally, through catheter.

PLATE I.

Subcutan., 0.8 cc. Massage.

FIG. 1 × ⅓.

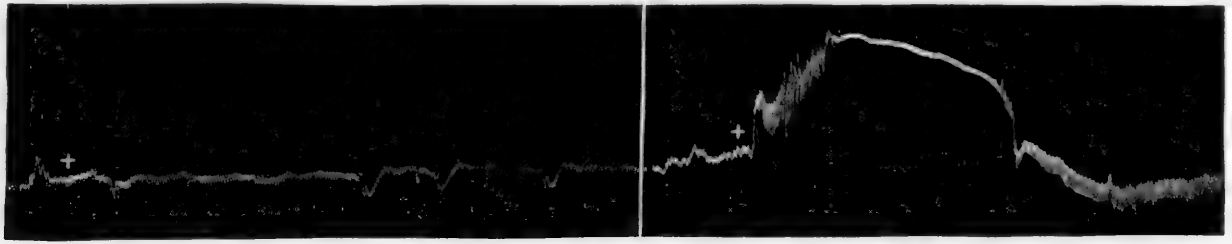


Subpectoral.
0.4 cc.

FIG. 2.

Intravenous.
0.3 cc.

FIG. 3.



Subcutan., 1 cc.
4.17.

FIG. 4 × ⅛.

Intramuscular, 0.8 cc.
4.26½.

FIG. 5 × ⅛.

THE COLOR REACTIONS OF NAPHTHAQUINONE SODIUM-MONOSULPHONATE AND SOME OF THEIR BIOLOGICAL APPLICATIONS.

By C. A. HERTER, New York.

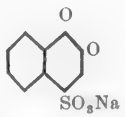
The extraordinary capacity of naphthaquinone-sulphonic acid to enter into reactions with the production of color was first recognized by Witt and Kaufmann,[1] who first prepared the substance by oxidation of amido-naphtha-sulphonic acid. The observations of Witt were recently considerably extended by Ehrlich and Herter,[2] who not only described a number of new color reactions, but also indicated various biological applications which promise to increase our physiological knowledge. Since the publication of these papers I have added a considerable number of new color reactions to those previously observed, and it is my purpose at present to describe some of these. I shall not undertake to discuss fully the chemistry of these reactions, which in many cases is still obscure. I shall, however, describe a number of reactions, selecting especially those which are characterized by sensitiveness, or by some quality which lends the reactions a degree of biological significance.

PROPERTIES OF THE SUBSTANCE.

The 1.2 naphthaquinone 4 sodium sulphonate is an orange-colored powder, which dissolves readily in water. In 95 % alcohol it is slowly and slightly soluble, solution being aided by heat; the solubility in absolute alcohol is still less. In acetone also it is moderately soluble. In ether, chloroform, carbon disulphide, benzene, and petroleum ether the substance is insoluble or very nearly so. The test of solubility in these cases was the failure to obtain any reaction with anilin. The substance is

[1] *Berichte d. Deutschen chem. Gesellschaft*, 1891–2, xxiv, 3157.
[2] *Zeitschr. f. physiolog. Chemie*, 1904, xli, 379.

readily reduced to the corresponding hydro-naphthaquinone compound by means of zinc dust and hydrochloric acid. When thus reduced a reaction with anilin can no longer be obtained. The ease with which reaction occurs in vitro is noteworthy from the fact that the substance undergoes reduction in the animal organism. Efforts to obtain reduction by means of alkaline solutions of glucose were unsuccessful, even when the mixture was subjected to boiling. Under these circumstances the solution becomes a very deep brown, which after boiling a few minutes gives place to a red tint. The reduced and colorless acid solutions of hydro-naphthaquinone sodium-monosulphonate are easily oxidized to the original compound on the addition of potassium persulphate. The proof of this is that the characteristic anilin compound is immediately formed on the addition of anilin to a solution of the hydro compound which has been subjected to oxidation. The chemical constitution of the naphthaquinone sodium-monosulphonate is indicated by the following graphic formula:



The addition of alkali to a watery solution of naphthaquinone compound leads to a gradual darkening of the solution. It is a property of quinones generally that their solutions darken on the addition of basic substances. Possibly this change is connected with tautomerism. This change is greatly accelerated by the use of heat. On rendering the solution acid by means of mineral or organic acids the solution becomes pale yellow. The chemical nature of these changes is not wholly clear. Many substances, when added to an alkaline solution of the naphthaquinone substance, give rise to a dark brown color like that just mentioned. I have found this to be the case in alkaline solutions of uric acid, and with solutions of caffein, xanthin, theobromin, alloxan, etc. I think it probable that the reaction represents merely an acceleration and intensification of the change which takes place when a fairly strong alkali is added to this substance. A similar

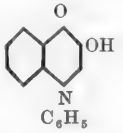
reaction can readily be demonstrated with normal human urine which has been rendered alkaline. If the naphthaquinone compound be added to alkalized urine there is a rapid darkening of the mixture. A dark-colored substance can be readily salted out by means of ammonium sulphate, but its nature is still uncertain. The intensity of the reaction appears to be little diminished by the previous removal of uric acid and other purin substances by means of ammonium salts.

TYPES OF COLOR REACTIONS.

The most striking example of the capacity of our substance to give rise to multitudinous color reactions is seen in the case of compounds which contain an aromatic primary amido group. The substances of this class which react may literally be numbered by the hundreds. For the most part the color reaction obtained in these cases is some shade of red or crimson, but in some instances the color is modified toward brown and usually deepens on the addition of alkali. A second important group, though one which is more limited in the number of its reactions than the preceding, illustrates the so-called acid methylene type. This term refers to such organic substances as possess a methylene (CH_2) group located between two negative radicals, such as CN, COO, C_2H_5, CO NH_2, C_6H_5, CO, $COCH_3$, etc. In all these cases the methylene group becomes labile, and takes on the capacity of making condensation products with our substance. In another group of cases we have reactions with a great variety of aliphatic primary amines, like methylamine and ethylamine. In a certain number of instances, also, we meet with color reactions due to secondary amines, both aromatic and aliphatic. Finally among the organic compounds we meet with some in which the color reaction can scarcely be regarded as depending on a true condensation but is rather to be ascribed to a process of oxidation. This appears to me to be the case with the green color produced by the action of the substance on resorcin.

It seems desirable to describe with some detail certain typical reactions belonging to these various groups of compounds.

Color Reactions of Primary Aromatic Amines.—One of the most striking reactions belonging to this group is that of anilin. This amine undergoes condensation with our substance in neutral solution and without the use of heat. The sensitiveness of the reaction is great, for in a solution of anilin of a concentration of one part in 256,000 parts water there are still indications of the characteristic fire-red precipitate which anilin yields. Even in concentration of one part in 1,000,000 an orange-red color is still perceptible. The constitution of the reaction product has been worked out and is expressed by the following formula.



In this reaction there is an elimination of sodium sulphite and one of the oxygen atoms in the naphthaquinone is replaced by the hydroxyl group.

The reaction with anilin has some biological interest, for it enables us to trace this substance in the organism. The organs of the animal poisoned with anilin are boiled, and a strong watery solution of naphthaquinone is applied to their cut surfaces. The presence of anilin is shown by the development of a red or pink color. Meta- and para-bromanilin give a red precipitate with our substance resembling that obtained with anilin, but there is a falling off in sensitiveness. Metabromanilin ($C_6H_3BrNH_2$) reacts in a solution of 1 part in 256,000; parabromanilin, in a solution of 1 to 16,000. In the case of chloranilin we see a similar difference in sensitiveness in the meta and ortho compounds, the former reacting in solutions of 1 to 250,000 and the latter in 1 to 32,000. The substitution of hydrogen by an alkyl group in the amido group of anilin gives us secondary amines of slight sensitiveness. Methylanilin ($C_6H_5NHCH_3$) and ethylanilin ($C_6H_5NHC_2H_5$) react in 1 part in 8000. The introduction of two alkyl radicals into the amido group of anilin causes complete failure to react. The introduction of a

nitro group into anilin also causes a diminution of sensitiveness, but metanitranilin ($NO_2\ C_6H_4\ NH_2$) still gives a red color reaction in 1 part in 512,000 and a precipitate in 1 part in 128,000. The ortho and para compounds are much less sensitive. The introduction of two negative radicals gives rise to a great diminution of sensitiveness. This is well seen in dinitranilin (1-2-4), [$(NO_2)_2\ C_6H_3\ NH_2$]. Three nitro groups, as in trinitranilin [$(NO_2)_3\ C_6H_2\ NH_2$], cause a failure to react, at least in neutral solution and in the cold. Methylbenzylanilin ($C_6H_5\ N\ CH_3\ C_6H_5\ CH_2$) and benzlyanilin ($C_6H_5\ NHCH_2\ C_6H_5$) are likewise negative. The introduction of a hydroxyl group into anilin in the para position gives us para-amidophenol ($OH\ C_6H_4\ NH_2$), a compound which possesses a considerable degree of sensitiveness. In neutral solution it gives with our substance a fine red, which on the addition of alkali changes to violet or purple. With the aid of alkali it is possible to detect the presence of 1 part of para-amidophenol in 250,000 of water. This reaction acquires a certain medical interest from the fact that para-amidophenol is given off from numerous anilin derivatives which are employed as antipyretics. The presence of para-amidophenol in the urine can be detected by means of the naphthaquinone reaction, but I have not had sufficient experience with the reaction to be able to state whether it possesses any advantage over the tests now in use. The naphthaquinone reaction, however, possesses the interest which arises from our being able to detect readily para-amidophenol in the tissues by means of it.

Of the aromatic diamines two toluylendiamines [$C_6H_3CH_3\ (NH_2)_2$] (1-2-4 and 1-3-4) may be mentioned. Both give red precipitates in neutral solutions. The latter compound (1-3-4) is considerably more sensitive.

As might be expected, toluidine ($CH_3\ C_6H_4\ NH_2$) and many of its derivatives enter into condensation with the naphthaquinone compound, and the same is true of xylidine [$(CH_3)_2\ C_6H_3NH_2$]. Toluidine gives a red precipitate which is detectable in 1 part in 250,000. Methyltoluidine, dimethyltoluidine, and diethyltoluidine fail to react. On the other hand various

nitro-toluidines react, and for the most part readily. The 1-3-6 compound can be detected in 1 part in 236,000; the 1-3-4 compound in a proportion of 1 part in 500,000. The remaining nitro compounds are less sensitive.

Other primary aromatic amines which react well with our substance are benzylamine ($C_6H_5\,CH_2NH_2$), benzylmethylamine ($C_6H_5\,CN_2\,NH\,CH_3$), a- and b-naphthylamine ($C_{10}H_7\,NH_2$), benzidine ($C_6H_4NH_2\,C_6H_4NH_2$), and phenetidine ($H_2N\,C_6H_4\,O\,C_2H_5$). The latter substance can be detected in 1 part in 1,000,000 in watery solution, and in consequence of this sensitiveness its distribution in the organism may be studied with little difficulty. It yields the red color characteristic of aromatic amines and their derivatives.

The sulphonic acid derivative of a-naphthylamine (1-4 naphthylamine-sulphonic acid—$C_{10}H_6\,NH_2\,SO_3H$) known as naphthionic acid, and much used in the production of dyes, makes salts which react with our substance. Other naphthylamine sulphonic acids (1-5, 1-6, 1-7, 2-5, 2-7) give similar red color reactions, but these substances vary in sensitiveness. The 1-8 compound gives an orange-red. All these substances should be tested in alkaline solution, that is, the solutions of their salts should be rendered alkaline. Naphthylamine disulphonic acid also reacts, but requires both heat and alkali. Amidostilbene disulphonic acid gives a violet color.

When a drop of a 2 % solution of naphthaquinone sodium-monosulphonate is added to a weak solution of phenylhydrazine ($C_6H_5\,NH\,NH_2$) in water, a purple violet color immediately results without the aid of heat. The addition of potassium hydroxide causes this color to fade. More concentrated solutions of phenylhydrazine yield a red color with our substance, the depth of the red increasing with the concentration of the naphthaquinone solution. One part of phenylhydrazine in 70,000 parts of water still gives the purple color. Benzylphenylhydrazine behaves in a similar manner. Phenylhydrazine oxalate gives a feeble color reaction, but the substance is only slightly soluble in water.

As is well known, anilin forms addition products with a num-

ber of acids. If we take a water solution of one of these addition products, e. g., anilin sulphate [$(C_6H_5NH_2)_2\ H_2SO_4$], it is found to react with the naphthaquinone compound, even in the presence of an excess of acid. The same is true of the hydrochloric acid compound.

Aliphatic Amines.—Among the aliphatic amines there are some which give distinct reactions with the naphthaquinone compound. In general, substances of this type yield a green color. It is of interest that ammonia itself, when mixed with a solution of naphthaquinone sodium-monosulphonate, gives a green color. Dilute solutions of ammonia give a brown color. As in the case of the aromatic amines, it is the primary amines that give the best reactions; thus ethylamine ($NH_2\ C_2H_5$) and methylamine ($NH_2\ CH_3$) react in the cold with a deep green color. Excess of acids causes a change to red. Dimethylamine [$NH\ (CH_3)_2$], on the other hand, reacts but feebly, with an orange-red color. Triethylamine [$N\ (C_2H_5)_3$] and trimethylamine [$N(CH_3)_3$] give no reaction, as might be predicted. Amylamine [$CH_3(CH_2)_3\ CH_2\ NH_2$] and hexylamine [$CH_3\ (CH_2)_4CH_2NH_2$] also react with the production of a deep green color. On the addition of acids the former changes to red-orange and the latter to red. Pentamethylendiamine, or cadaverine ($NH_2\ CH_2\ CH_2\ CH_2\ CH_2\ CH_2\ NH_2$), also gives a green color reaction, which changes to red on the addition of acids. I had no tetramethylendiamine (putrescine) at my disposal, but it appears safe to predict that it will be found to react.

Aromatic and Aliphatic Amidoacids.—It has already been stated that substances containing the amido group are apt to react with the naphthaquinone compound. There is, however, a great difference between the behavior of the amidoacids and the acid amides. The former, as a rule, react readily and give a red color or some shade of brown. The acid amides generally fail to react. Among the aromatic amidoacids may be mentioned in this connection the amidobenzoic acids ($C_6H_4\ NH\ _2CO\ OH$), methylamidobenzoic acid (para), and amidosalicylic acids. Anthranilic acid (o-amidobenzoic acid) reacts in 1 part in 250,000; and sulphanilic acid ($C_6H_4\ NH_2\ SO_3$) is not less sensitive. Of

physiological interest is the fact that amidococaine is a reacting substance. Among the aliphatic amidoacids we have glycocoll (amidoacetic acid, $NH_2\,CH_2\,COOH$), alanin (amidopropionic acid, $NH_2\,C_2H_4\,CO\,OH$), and leucin ($C_5H_{10}\,NH_2\,CO\,OH$).[3]

In contrast to the lability of the amidoacids stand the various acid amides: thus, salicylamide ($C_6H_4\,OH\,CO\,NH_2$), benzamide ($C_6H_5\,CO\,NH_2$), phthalamide [$C_6H_4(CO)_2(NH_2)_2$], thiobenzamide ($C_6H_5CS\,NH_2$), toluylamide ($CH_3\,C_6H_4\,CO\,NH_2$), acetamide ($CH_3\,CO\,NH_2$), proprionamide ($C_2H_5\,CO\,NH_2$), lactamide ($CH_2\,CH\,OH\,CO\,NH_2$) fail to react, or react so feebly as to be hardly distinguishable from the controls. Carbopyrrolamide ($C_6H_4N\,CO\,NH_2$) and cyanacetamide ($CN\,CH_2\,CO\,NH_2$) give color reactions in alkaline solutions, the former a green, the latter a purplish red, but these reactions depend on the presence of the pyrrol nucleus and the acid methylene group respectively.

Asparagin ($NH_2\,CO\,CH_2\,CH\,NH_2\,CO\,OH$) reacts with the red-brown color noted in the reactions of the acid amides. Tyrosin ($OH\,C_6H_4\,CH_2\,CH\,NH_2\,CO\,OH$) gives a reddish yellow color deepened by alkali and intensified by acid. Sarcosin ($CH_3\,NH\,CH_2\,CO\,OH$) gives an orange-red in water solution, the color deepening on the addition of alkali.

The color reactions of the amidoacids with the naphthaquinone sodium-monosulphonate suggest the possibility of our being able to follow these substances (at least as a group) in their origin from proteid in the intestine and during their absorption and further distribution.

It should be mentioned here that while carbamide [urea—$CO(NH_2)_2$] and biuret ($NH_2\,CO\,NH\,CO\,NH_2$) do not react, the urea derivatives semicarbazid ($NH_2\,CO\,NH\,NH_2HCl$) hydrochloride and thiosemicarbazid ($NH_2\,CS\,NH\,NH_2$) give red colors with the sulphonate. The latter substance still reacts

[3] Not without interest for physiology is the fact that the hexon bases (diamidoacids) enter into color reactions with the naphthaquinone compound. Preparations of histidine chloride, lysin picrate, arginine nitrate, and ornithin were submitted to me by Dr. Wakeman, who prepared them in Kossel's laboratory. Of these substances, ornithin was found to react most readily. Dilute alkaline solutions of histidine chloride gave an amethyst color. The other hexon bases give the reddish colors usually observed with amidoacids.

when present in the proportion of 1 part in 200,000 if alkali be present. The red which results is a fine crimson, if the naphthaquinone be used in dilute solution. It is destroyed by acid. Diphenylcarbazid also reacts.

Color Reactions with Heterocyclic Compounds.—Among the heterocyclic compounds there are some which give with the naphthaquinone sodium-monosulphonate highly characteristic color reactions of considerable chemical and physiological interest. We have to consider especially pyridine and piperidine (and their derivatives), pyrrol, thiophene, and the pyrazaol derivatives, and finally indol and skatol.

Pyridine (C_5H_5N) either gives no color reaction at all with our substance, or gives a reaction so slight that it is with difficulty distinguishable from a control.[4] On the other hand, the hexahydro compound, piperidine ($C_5H_{10}NH$), gives in water solution a fine scarlet color which gradually fades. The reaction is much hastened by heat. The color is destroyed by alkalies and acids. This reaction is a moderately delicate one, the color being still discernible on the addition of our substance to a solution of 1 part of piperidine in 32,000 parts of water. This reaction with piperidine is probably to be referred to condensation with the labile imide (NH) group contained in this substance, but the chemical nature of the newly formed compound has not been studied.

Of the derivatives of pyridine, the monomethyl compound, picoline, is also negative as regards color reaction. The dimethyl, trimethyl, and tetramethyl pyridines, known respectively as lutidine, collidine, and parvuline, each yield scarlet precipitates with the naphthaquinone compound. These reactions take place most readily in water solutions. Of the derivatives of piperidine, a-pipecolin ($C_5H_9CH_3NH$) gives a fine red, which is destroyed by excess of alkalies; this reaction is facilitated by heat, but takes place in the cold. A-propyl piperidine [coniine ($C_8H_{17}N$)] reacts to our substance to make a deep red color, which is destroyed by acids, but not so readily by alkalies. Another alkaloid, nicotine ($C_{10}H_{14}N_2$), closely related to piperidine,

[4] Kahlbaum's pyridine gave no reaction.

gives a more characteristic reaction with the naphthaquinone sodium-monosulphonate. Even with a small amount of the sulphonate the water solution of nicotine yields a yellow olive-green, which gradually changes to reddish brown. This color is destroyed by acids and alkalies. The reaction is hastened by heat.

Of the members of the pyridine carbonic acid group, the b-compound, nicotinic acid (N C_5H_4CO OH), is the only one which has yet been tried and it proved negative. The members of the furfurane group, including furfurane, furfurole, and pyromucic acid likewise failed to react. On the other hand, pyrrol (C_4H_4NH) was found to give a beautiful and characteristic reaction. If a solution of pyrrol of moderate concentration be treated with one drop of a 2 % solution of the naphthaquinone compound, the solution gradually assumes a pink color which soon changes to purple or violet. The reaction is accelerated by boiling the pyrrol solution before adding the reagent. If the reaction be carried on with the aid of potassium hydroxide, the sequence of colors is somewhat modified, the red tints being prominent at first and gradually changing to purple or violet, if the excess of alkali be considerable. After the color reaction has been obtained with the naphthaquinone, either in watery or distinctly alkaline solution, the addition of acids in excess occasions the development of a yellowish green tint which, after a time, fades. The use of a strong acid, like hydrochloric, occasions the immediate development of the green tint. Regarded as a method of distinguishing pyrrol, this color reaction cannot be said to be remarkably delicate, since in greater dilution than 1 part in 4000 the colors are no longer well marked. Nevertheless, the naphthaquinone color reaction for pyrrol may be regarded as a contribution to our means of detecting this substance. Looked at as a test for the naphthaquinone sodium-monosulphonate, the reaction described is of considerable interest, since even so small an amount as 0.8 of one milligram of our substance yields the typical color reaction with a moderately concentrated pyrrol solution. Of course, we have in anilin a substance with which it is possible to detect small amounts of naphthaquinone sodium-

monosulphonate, but if we take the urine of a rabbit, to which the naphthaquinone compound has been administered, this urine fails to react with a solution of anilin. On the other hand, I have found that such an urine, added to a pyrrol solution which has been boiled, yields the typical colors described, or at least gives a green coloration on the addition of acid. In other words, I have employed pyrrol successfully for the detection of naphthaquinone sodium-monosulphonate in the urine when other reagents have failed. The colored substances formed by reaction of pyrrol with our sulphonate in alkaline solution is readily reduced to a colorless solution by the addition of glucose to the boiling mixture. On the addition of potassium persulphate to the colorless mixture, the leuco body is apparently oxidized to the original colored substance.

The iodine derivative of pyrrol known as iodol [tetraiodopyrrol — (C_4I_4NH)], if dissolved in alcohol and water, reacts slowly with the sulphonate. A blue color results. Alkali should be used in order to get this reaction.

We have in piperidine and its homologues examples of the reaction of the imide (NH) group with the naphthaquinone sulphonate. In pyrrol we have an example of a similar reaction, and this is probably true of pyrrolidine ($C_2H_4\ C_2H_4\ NH$) or tetra hydropyrrol (tetramethyleneimide), and of pyrrolidine-a-carbonic acid [5] ($C_2H_4\ C_2H_3\ CO\ OH\ NH$), a substance which has recently assumed a physiological interest as a cleavage product of proteid material. The discovery of the pyrrol reaction which I have described led me to search for other compounds containing the imide group, and an interesting example of such a substance was found in the cyclic alkylen imide known to the medical profession as piperazine ($NH\ C_2H_4\ C_2H_4\ NH$) and to chemists as diethylenediamine. This substance contains two imide

[5] Dr. Flexner was so kind as to furnish me with the active and inactive copper salt of a-pyrrolidine-carbonic acid prepared by him. A solution of this active salt was not changed by the addition of a drop of the 2 % naphthaquinone solution, but on the addition of potassium hydroxide a well-marked red soon developed, even in the cold. The solutions of the inactive copper salt reacted more slowly under the same conditions and perhaps less fully, an amethyst color being developed as an intermediate stage on the way to brownish red.

groups, and may be looked upon as piperidine, in which the methylene group occupying the para position to the imide group has been replaced by a second imide group. It was interesting to observe that this substance in dilute watery solution reacts very readily in the cold, with the naphthaquinone sodium-monosulphonate with the production of a fine red.[6] No alkali is necessary for the production of this reaction. I have not been able to learn that there is any other color reaction for piperazine. It should be observed further that piperazine reacts much more sensitively than piperidine, which can doubtless be explained through the presence of the second imide group. It will be seen presently that there are two other heterocyclic derivatives, i. e., indol and skatol, which react by virtue of their imide groups. On the other hand, phthalimide [$C_6H_4(CO)_2$ NH] and carbazol (C_6H_4 NH C_6H_4) gave no reactions.

Thiophene.—It may be remarked here that the sulphur homologue of pyrrol, known as thiophene (C_4H_4S), reacts with the naphthaquinone substance in hot alkaline solution, showing a delicate purple which is destroyed by excess of acid.

Pyrazol Derivatives.—In connection with the heterocyclic compounds, I may refer to the reaction noted in some pyrazol (HN N : CH CH CH) derivatives. One of the most important of these is phenylpyrazolone (HC N : NC_6H_5 CO CH_2), which reacts green with our substance and alkali, but soon changes to blue, and on boiling becomes greenish blue. Excess of acids causes a change to yellow-red. Methylphenylpyrazolone reacts similarly. Dimethylphenylpyrazolone (CH_3 C N CH_3N C_6H_5CO CH), or antipyrine, does not react, and negative results are also obtained with solutions of dimethylamidodimethylphenylpyrazolone or pyramidon. But these antipyretic drugs can readily be forced to unite with the naphthaquinone sodium-monosulphonate. If we dissolve antipyrine in water it can readily be converted into the green nitroso-antipyrine by the use of sodium nitrite and hydrochloric acid. The nitroso-antipyrine may now readily be reduced to the amido compound by means of zinc and hydro-

[6] Urine to which a small amount of piperazine has been added readily gives this reaction.

chloric acid. But we have seen that amido derivatives of aromatic compounds generally react with our substance. The present instance is no exception, and the typical red reaction is readily obtained. Pyramidon gives similar results.

CONDENSATION PRODUCTS OF THE BENZENE NUCLEUS WITH HETEROCYCLIC NUCLEI.

In this group we have to consider certain quinoline (C_9H_7N) derivatives, and the far more important substances, indol and methylindol, or skatol. Of substances pertaining to the quinoline group, it was found that quinoline and isoquinoline failed to react with the naphthaquinone compound. Oxyquinoline, however, in alkaline solution gave a deep olive-green or green-brown fluid which, on dilution with water, brightened to green. The color is destroyed by acids. Paratolu-quinoline [$C_9H_6(CH_3)N$] in alcoholic solution was found to give a deep red-brown coloration on the addition of a solution of naphthaquinone sodium-monosulphonate. This color was not damaged by moderate excess of acids or moderate excess of alkali.

Of the various color reactions which have been brought to light through experiments with the naphthaquinone derivative, none are of greater physiological importance or of more interest to the chemist than those which relate to the behavior of indol and skatol. Although the color reaction obtained by combining a solution of indol with the naphthaquinone derivative was known to Professor Ehrlich, he never obtained an opportunity to study it, and no mention was made of it in the publication to which I have referred.

The course of the color reaction between indol or skatol and the naphthaquinone compound varies somewhat with the conditions under which the test is carried out. For this reason it is necessary to observe rather closely certain details in order to obtain comparable results. If we add to the fairly dilute solution of indol in water (say 1 part indol in 50,000 of water) 1 drop of the 2 % solution of the naphthaquinone sodium-monosulphonate, no reaction occurs. On the addition of a drop of 10 % solution of potassium hydroxide there gradually develops

a blue or blue-green color, which fades to green on the addition of an excess of alkali. On rendering acid the green or green-blue solution, the fluid assumes a pink color. The development of the color reaction is markedly hastened by heat. If, instead of adding the alkali to the indol solution in the test-tube after the addition of the naphthaquinone solution, one adds the alkali previously to the introduction of our substance, the course of the reaction is somewhat different, provided the concentration of the indol solution be somewhat greater than that already mentioned, and provided also that the reaction be carried on with the aid of heat. Under these circumstances the blue color develops and deepens, but in a short time it becomes evident that the precipitation of the new color compound is taking place. At first the indol compound is separated in fine particles which coalesce to form larger ones, and which possess a spongy appearance, and after a time rise to the surface, leaving the faintly tinted mother liquor. If particles of this blue sponge-like substance be examined with the aid of a microscope, it is found to consist entirely of well-defined acicular crystals, resembling pine-needles in shape and closely felted together. These crystals are blue, and have a diameter of about one micron and a length of from fifteen to forty microns. They are very slightly soluble in water, and considerably more soluble in alkali. The chemical nature of this felt-like substance is not at present wholly clear, but some facts regarding it have been acquired. A considerable quantity of the new compound was made by the method just outlined, collected on a filter, and washed with water. The material thus obtained was dried to constant weight and then subjected to a nitrogen determination by Dr. Wakeman. The proportion of nitrogen contained in the molecule of the new substance was such as would correspond closely to a compound formed by the union of one molecule of naphthaquinone sodium-monosulphonate with two molecules of indol.[7] This result points

[7] The percentage of nitrogen in a compound consisting of two molecules of indol and one of naphthaquinone monosulphonate is 5.669 %. No allowance is here made for the elimination of one molecule of water, which must occur if the above assumption as to the constitution of the new compound be correct. Making this correction the percentage of nitrogen is 5.88 %. The percentage

to the condensation of one of the carbonyl groups in the naphthaquinone compound with the imide group of two indol molecules, as in the condensation of quinone and hydroxylamine to form quinoneoximes. It is difficult to see how the condensation can take place with the elimination of the sulphonic acid group, as occurs in the case of the formation of the naphthaquinone anilid already mentioned.

A highly interesting feature of the condensation product of indol and the naphthaquinone compound is its solubility in chloroform, acetone, and other solvents, with the production of an intense red color. If we cause the formation of the blue color by bringing together indol and our substance in the manner described above, the blue color can be quickly removed by shaking the fluid with chloroform. As the chloroform grows pinkish-red, the blue color disappears from the aqueous solution. This property is of considerable importance in testing for indol, as it serves to distinguish the indol reaction from other reactions which yield a similar color. The relation between the red color of the solvent and the blue color of the compound can be very strikingly exhibited by means of the following experiment. A small quantity of the washed reaction product of indol and the naphthaquinone derivative is dissolved, with or without the aid of heat, in acetone. The color of the acetone is at first red and deepens to purple-red. On diluting with water and adding potassium hydroxide the solution grows blue, and this blue color can readily be transformed to red by shaking out with chloroform, as already described.

The behavior of skatol is very similar to that just described for indol. Strong solutions of skatol yield a blue color on the addition of the naphthaquinone, provided they have been already rendered alkaline. Weak solutions of skatol do not yield a blue color, but give rise to a distinct violet or purple hue. This is the most important feature in the distinction of skatol from indol.

actually found in the new compound was 5.819 %. A further confirmation of the correctness of the above supposition as to the nature of the compound exists in the fact that it contains sulphur, which it could not contain were the condensation to occur as in the case of the union with aniline.

By means of the procedure which has already been described for indol, it is possible to separate the reaction product of skatol and naphthaquinone sodium-monosulphonate. The crystals in this case are of the same form as those described in connection with the indol compound, and are similarly arranged. They are, however, smaller and are violet in color. They are soluble in acetone and chloroform, with a resulting brilliant red color, like that described for the solution of the indol compound. The violet color obtained from skatol and the naphthaquinone derivative can therefore be washed out of ordinary alkaline aqueous solutions by means of chloroform.

The reactions of indol and skatol with the naphthaquinone compound are delicate, and it is possible to detect these substances in alkaline aqueous solutions of about one part in one million parts of water, if suitable precautions are taken in making the test.

Reactions of Phenols.—A considerable number of phenols react with our substance, but only a few will be mentioned here. Common phenol (C_6H_5OH) reacts in alkaline solution with a blue-green color. Orthocresol ($CH_3\,C_6H_4OH$) likewise reacts green, but para-cresol is insensitive, and dinitrophenol [(NO_2)-C_6H_3OH] also fails to react, the acid groups here interfering. Trinitrophenol [$(NO_2)_3C_6H_2OH$], (picric acid), is also negative. Thymol ($C_3H_7\,C_6H_3CH_3OH$) gives a blue-green. Of the dihydroxyphenols resorcin [$C_6H_4(OH)_2$ 1:3] gives a green in alkaline solution, which is fairly sensitive (in 1 part in 30,000 of water). Under certain conditions it is possible to obtain a violet color after the appearance of the olive-green. Hydroquinone [$C_6H_4(OH)_2$ 1:4] reacts brown in the presence of alkalies. Pyrocatechin [$C_6H_4(OH)_2$ 1:2] in alkaline solution gives with the sulphonate a red or olive color (according to the conditions of the reaction), which probably depends on oxidation. A-naphthol gives a green color with our sulphonate, but the reaction is not delicate; b-naphthol does not react. Of the trihydroxyphenols, phloroglucin [$C_6H_3(OH)_3$ 1:3:5] gives a blue in sodium carbonate solution, which alters in a few minutes to blue-violet and on heating gives a dark blue. On the addition of acids the

color changes to yellow. It is a general rule that acids bring about the decoloration of the phenolic color compounds of our substance, a yellow or yellow-red fluid usually remaining.

Pyrogallol (1:2:3) in alkaline solution changes to red on the addition of the sulphonate, but I attribute this to a further oxidation and not to a condensation, for the same result is obtained with oxidizing agents. I have already mentioned that the reaction with resorcin probably depends on oxidation. Oxyhydroquinone [$C_6H_3(OH)_3$ 1:3:4] yields a red-brown in alkaline solution, and is the most sensitive of the three trihydroxyphenols.

Reactions Based on the Acid Methylene Group.—As already stated there are a number of naphthaquinone reactions which depend on condensation with the methylene (CH_2) group. It is, however, chiefly in the case of methylene groups which lie between two negative radicals that these reactions take place. For our present purpose it is not necessary to describe these reactions in detail. The following substances may, however, be mentioned as examples of bodies which enter into reactions of this type: acetylacetone ($CH_3\,CO\,CH_2\,CO\,CH_3$), benzoylacetone ($C_6H_5\,CO\,CH_2\,CO\,CH_3$), acetonedicarbonic-ethylester ($CO_2C_2H_5\,CH_2)_2\,CO\,CH_2$, desoxibenzoin ($C_6H_5\,CO\,CH_2C_6H_5$), cyanacetamid ($CO_2NH_2CH_2\,CN$), acetacetic-ethylester ($CH_3\,CO\,CH_2\,C_2H_5\,CO_2$), and benzoylaceticester ($C_6H_5\,CO\,CH_2\,C_2H_5\,CO_2$). Of these substances acetylacetone and benzoylacetone give red-brown colors. Acetaceticester and acetonedicarbonic ethylester yield orange-red tints. Cyanacetamid gives an immediate red, which deepens in one or two minutes to purple-red, but on boiling develops into a deep red-violet. All reactions referred to in this section are developed in alkaline solution.

Compounds of Hydrocyanic Acid.—The majority of the organic compounds of hydrocyanic acid which have been examined failed to react with naphthaquinone sodium-monosulphonate; thus, proprionitrile (C_2H_5CN), butyronitrile ($C_3H_7\,CN$), mandelicacidnitrile ($C_6H_5CHOHCN$), benzaldehydecyanhydrine ($C_6H_5CHOHCN$), acetonecyanhydrine (C_2H_6COHCN), aldehydecyanhydrine ($CH_3CHOHCN$), and metatolunitrile ($CH_3C_6H_4CN$) failed to react. Benzylcyanide ($C_6H_5CH_2CN$) gave a

red-brown reaction with alkali, but the reaction was feeble. Acetonitrile (CH_3CN) gives a similar reaction. Malonitrile [$CH_2(CN)_2$] gives a green color in the cold without alkali. The addition of caustic potash to the green solution gives a deep red which fades slowly. A-naphthonitrile ($C_{10}H_7CN$) reacts with a deep red color in the presence of potassium hydroxide. Orthotolunitrile and paratolunitrile ($CH_3C_6H_4CN$) give red reactions with alkali. It may be mentioned in this connection that guanidine ($CNH<\begin{matrix}NH_2\\NH_2\end{matrix}$) in alkaline solution gives a violet color with the naphthaquinone compound, while amidoguanidine reacts red. Rhodaninic acid in alkaline solution gives a deep violet.

Reactions with Compounds of the Purin Base Type.—Mention has been made already of the fact that uric acid, xanthin, caffeine, alloxan, etc., in the presence of an excess of alkali cause a rapid browning of the naphthaquinone compound. The nature of the change which the reagent undergoes is not known, but it appears to be of the same nature in all these cases. It appears, moreover, to consist in an accentuation and acceleration of the change which occurs when an alkaline solution of the monosulphonate is permitted to stand.

A reaction of a different sort is observed in the case of a substance related to the purin bases, namely, murexid, the ammonium salt of purpuric acid [$C_8H_4(NH_4)N_5O_6$]. This body contains five imide groups, four of which pertain to the two alloxan radicals which are united through the fifth imide group. It seems likely that it is the presence of this fifth imide group which causes the murexid reaction to differ from the reactions above mentioned.

If one adds to a water solution of murexid in a test-tube one drop of a 2 % solution of our monosulphonate, only a slight yellow coloration occurs in the fluid. But on the addition of three or four drops of a 10 % solution of potassium hydroxide, there gradually develops (without the aid of heat) a fine violet color, which ultimately deepens considerably and then fades. The addition of an excess of naphthaquinone leads to the pro-

duction of a red fluid which may deepen to brown, and which on dilution may assume a green tinge.

I have not been able to find any description of another color reaction for murexid.

Reactions of Substances containing Sulphur.—It was found in the course of experiment that many substances containing sulphur react with the naphthaquinone compound in a characteristic way. The addition of the sulphonate to a solution of sodium sulphide causes the immediate formation of a dark brown (sometimes black) reaction product, which after a few seconds disappears, leaving the solution light red. This behavior is probably due to reduction. By the use of a great excess of the naphthaquinone compound it is possible to obtain a permanent dark brown color. Similar results are obtainable by passing a stream of hydrogen sulphide through a naphthaquinone solution and then adding alkali.

Observations were made on several mercaptans, including benzylmercaptan ($C_6H_5 CH_2 SH$), butylmercaptan (C_4H_9SH), and ethylmercaptan (C_2H_5SH). All gave a brown color similar to that which was obtained in the case of inorganic sulphides. It was noticed that when acid was added in excess to a solution in which the brown reaction product had been formed, the brown color gave way to a yellow-green fluid exhibiting opalescence. A similar but less pronounced decolorization opalescence was noticed in the case of ethylmercaptan. Slight opalescence was still noticeable in a water solution of one part of the mercaptan in about 20,000 of water. I think this behavior of ethylmercaptan may prove useful to chemists as an adjuvant to the usual test with a mercuric compound. The opalescence is apparently due to the separation of sulphur. This separation is more marked in solutions of sodium sulphide and ammonium sulphide.

Various sulphur derivatives of urea give a brown color with our sulphonate, the color disappearing rapidly unless a considerable excess of the naphthaquinone compound has been used. But these urea derivatives react only after the employment of heat and alkali.

Various proteids and allied bodies (fibrin, caseine, gelatin) behave like the thioureas, and it may be surmised that their capacity to give this reaction depends on the presence of sulphur in some cleavage product.

The thioureas tested were thiourea [$(NH_2)_2CS$], phenylthiourea ($CS\ NH_2\ NH\ C_6H_5$), and allylthiourea (thiosinamine—$CS\ NH_2\ NH\ C_3H$).

In testing for proteids and for thioureas it is of the first importance to make a careful control observation with the naphthaquinone compound, for this gives a brown-red color when boiled with alkali. If we take two test-tubes, one containing a hot-water solution of a definite (considerable) quantity of potassium hydroxide alone, and a second containing proteid which has been boiled with the same amount of potassium hydroxide, and add to each tube one or two drops of the naphthaquinone solution, the reaction of the proteid is obvious and is especially marked in the first seconds. But if the comparison be carelessly made, with excess of the reagent, the reaction may be masked. Red succeeds the original evanescent brown. The proteids give the sulphur (?) reaction even after the boiled solution has been cooled.

Reactions of Proteids.—No thorough study of the reactions of the sulphonate with proteids has been made, but it is certain that such reactions occur. Edestin furnished me by Mr. F. Underhill caused a reddening of the naphthaquinone solution when treated with potassium hydroxide. Merck's mucin yielded a dark brown, and casein (impure) behaved similarly, the color being transient. Witte's peptone (chiefly albumoses) caused some browning of the reagent. Small quantities of proteid have little effect. Crystalline leucylglycyl prepared by Dr. Flexner in the laboratory of Emil Fisher gave a green color in the presence of the monosulphonate, but heat and alkali were required to develop this. In all experiments with proteids it is of the utmost importance that the controls be carefully made, for heat and alkali act on the mono-sulphonate to cause changes in the color of this substance, due to unknown changes in constitution. A study of the proteid group reactions is now in progress.

Unclassified Reactions.—The list of subjects already mentioned as reacting with our sulphonate to yield color reactions may perhaps be fairly regarded as representative, but it certainly is not exhaustive. Reactions have been observed with a number of substances which belong in the categories here adopted, and it is safe to predict that many more will be found in time. Some substances give reactions which it is not possible to classify at present. Thus nitrourethane (NO_2 NH $CO_2C_2H_5$) gives a blue-violet in alkali, trinitrotoluol [CH_3 C_6H_2 $(NO_2)_3$] a brown-red, pyrotartaric acid ethylester (CH_3 CO CO_2 C_2H_5) a deep green, acetone (CH_3 CO CH_3) a pink color, and Michler's ketone or tetramethyldiamidodiphenylketone [$(CH_3)_2$ N C_6H_4 CO C_6H_4 N $(CH_3)_2$] a deep red in alkaline hot alcoholic solution. This reaction is of no diagnostic value and is difficult to demonstrate.

ON THE METHOD OF USING THE NAPHTHAQUINONE COMPOUNDS.

As much depends upon the way in which our compound is used in making tests, it is desirable in examining any substance with a view to finding whether it gives a color reaction to follow a definite order of procedure and to observe certain precautions. If the substance to be examined is an acid, its solution should be neutralized before adding the naphthaquinone, as a free acid is apt to decolorize any colored body that may be formed. On the other hand, if the solution to be tested is naturally alkaline to litmus, or has been rendered alkaline by the addition of an alkaline carbonate or hydroxide, the important influence of alkali in deepening the color of the naphthaquinone solution must be kept in mind. It is necessary in such instances to make a control observation on a solution of the reagent, to which has been added an amount of alkali comparable to that used in the test. Similarly it is essential to remember that even the weak solutions of naphthaquinone sodium-monosulphonate are greatly deepened in color when boiled in the presence of alkali. A moderately concentrated watery solution of naphthaquinone sodium-monosulphonate assumes a deep red-brown color on boiling with potassium hydroxide. It is therefore important to take account both of the quantity of alkali used, and of the concentration of

the reagent. The nature of the alkali is not a matter of indifference, for many substances, which give a reaction with the naphthaquinone compound in the presence of caustic potash, do not give this reaction in the presence of sodium carbonate.

When the substance to be tested has been brought into solution in water a few drops of a 2 % aqueous solution of the naphthaquinone compound are added. If no color appears a few drops of a 20 % solution of caustic potash are introduced. If there is still no color reaction the mixture in the test-tube may be boiled, and the color which develops is compared with that of the control, made as above mentioned. The effect of acetic acid and of mineral acids should be tried separately upon the alkaline solution as well as upon the neutral mixture. It is not always a matter of indifference whether we add the alkali before or after the introduction of the naphthaquinone compound. Thus in preparing the reaction product of our substance with indol, it was found best to render the indol solution alkaline before introducing the naphthaquinone compound. The concentration of the substance to be tested sometimes exerts a distinct influence on the result of the test. For example, a strong solution of resorcin to which a few drops of a 20 % solution of caustic potash has been added assumes a red color, and this color remains on dilution with water. The addition of potassium hydroxide, however, brings out the characteristic green color. The nature of the solvent must be taken into consideration at times, and the same solvent must be used in making the control observations. In the case of solutions where acetone or a mixture of acetone and water is used, it must be remembered that the naphthaquinone reagent gives a ruby-red or pink color with this ketone. Further details need not be mentioned, as they will suggest themselves.

BIOLOGICAL APPLICATIONS.

In consequence of the properties which have already been described or mentioned, the naphthaquinone sodium mono-sulphonate possesses a number of biological applications. The number of applications known to us at present is few com-

pared to those which experiment will show us to exist. What we know at the present time of the biological uses of the substance can be best discussed under three headings; first, the distribution of the aromatic compounds in the living organism; second, the occurrence of syntheses in the living organism; and, finally, the action of certain naphthaquinone color compounds in the body.

DISTRIBUTION OF AROMATIC COMPOUNDS.

The study of the distribution of those aromatic compounds which react readily with naphthaquinone sodium-monosulphonate is only in its first stage of development; but the experiments already made show that by means of our substance there is much to be learned regarding the relation of the distribution of substances and their chemical constitution. The few experiments which have been made up to the present time relate especially to the distribution of antipyretic drugs. In this class we have the derivatives of anilin, like phenetidine, phenacetine, acetanilid, and paraamidophenol, and, furthermore, pyrazolone derivatives, including antipyrine and dimethylamidoantipyrine or pyramidon. In experiments made recently with Mr. Frederic Bartlett it was found that these various substances could be detected without much difficulty in the liver and kidneys of rabbits a few hours after the administration of fairly large doses. In some instances the quantity found in given weights of liver pulp approximated the quantities obtained from the treatment of the nervous system, if one may judge from the intensity of the color reactions. The preparation of the liver pulp differed somewhat according to the substance sought. Alcohol was used in the extraction of substances which, like phenacetin and acetanilid, are not readily soluble in water. The proteids were precipitated in most instances by means of acetic acid, the filtrate being concentrated and neutralized before the application of the naphthaquinone test. In the case of phenetidine and paraamidophenol which react directly, no further steps were necessary before applying the reagent. Phenacetin and acetanilid, however, do not react directly, and it was necessary to decompose

the molecule by boiling with dilute sulphuric acid before applying the naphthaquinone test. The anilin sulphate thus formed reacts with the sulphonate.

The detection of antipyretics in the cells of the liver, freed as far as possible from blood, is an observation of some clinical interest, because it shows that other parts of the body besides the nervous system take up the substances. This fact should awaken us to the possibility of damaging other parts of the organism than the nervous system, by the indiscreet, long-continued use of analgesic antipyretic drugs.

Among the substances which it would be of interest to trace in the organism by means of our compound may be mentioned the derivatives of salicylic acid and cocaine; whereas salycilamid does not react with the naphthaquinone compound, amidosalicylic acid enters into a color reaction and can probably be traced by means of this. Cocaine does not react with our substance, but on the introduction of the amido group gives us a substance which reacts similarly to aromatic amines. It is true that the anæsthetic action of cocaine is somewhat diminished through this substitution, but since this physiological effect is not destroyed, a knowledge of the distribution of amidococaine in the organism might prove of some interest. It seems reasonable to believe that by the aid of our sulphonate it would be possible to trace the passage of many organic substances into the interior of the eye.

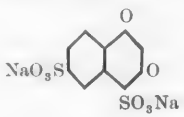
A promising field of investigation appears to me to be the excretion of aromatic compounds through the bile. Of the many substances which react with our sulphonate there are certainly a number, and probably many, which find their way into the bile. The selection from these of the most highly bactericidal and least toxic for the mammalian organism might prove of use in preventing and combating infections of the bile passages.

EXPERIMENTAL SYNTHESES IN THE LIVING ORGANISM.

It is natural that so reactive a substance as the naphthaquinone sodium-monosulphonate should have led to endeavors to bring

about syntheses with the living body. Experiments having this end in view were begun in the laboratory of Professor Ehrlich, and some of these were referred to in the conjoint publication already mentioned. It will not be out of place to refer here to these experiments, and to certain additional ones that have since been undertaken, although the results that have been obtained up to the present represent only a partial degree of success.

Observations made by means of intravenous infusion of the naphthaquinone sodium-monosulphonate gave results that were so little encouraging, that trials were made with the corresponding disulphonate. This substance possesses a second sulphonic acid group in the 6 position, as the following formula indicates:

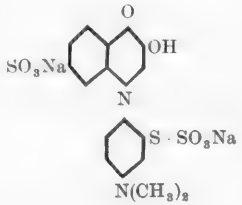


This secondary sulphonic acid group is not eliminated in the course of ordinary condensations with other substances. The disulphonate is less toxic than the monosulphonate, and confers increased solubility not only on the substance itself, but on its reaction products. The dyes formed through the reactions of the disulphonate assume the character of acid dyes.

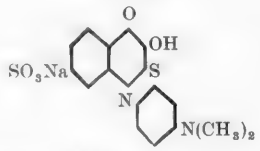
The first experiments undertaken were made with anilin with the intention of developing a neutralizing antitoxic action. This undertaking was, however, wholly unsuccessful, for although the red product of condensation could be detected in the bile, there was no evidence of an actual synthesis in the living cells. This fact is in itself of considerable physiological interest, for it indicates that certain cells, like those of the liver, are capable of holding apart substances in spite of the fact that they possess a strong chemical affinity for one another. The explanation of this probably lies in the different destinations of the two substances in the cell territory. This idea seems not improbable when one reflects that the different intracellular enzymes must be conceived to operate in physiologically separate portions of individual cells. The proof that both substances exist side by side

ON THE BEHAVIOR OF SOME PRODUCTS OF THE NAPHTHAQUINONE SULPHONATES WITH DIMETHYLPARAPHENYLEN-DIAMINETHIOSULPHONIC ACID.

When the naphthaquinone sodium-disulphonate is brought into relation with a solution of dimethylparaphenylendiaminethiosulphonic acid in equi-molecular quantities, there occurs, under suitable conditions, a synthesis, which results in a violet dye possessing the following constitution.



which is easily converted into a thiazine derivative



with the elimination of sulphurous acid. This dyestuff, which is known to the trade as indochromogen S, forms a readily soluble alkali salt, a solution of which possesses only a moderate grade of toxicity for rabbits, intravenously infused. Thus from 50 to 80 cubic centimetres of a 2 % solution may be infused intravenously at the rate of 2 cc. per minute, usually without bringing about death during the infusion. If we examine an animal which has been infused in this manner with a solution of indochromogen S, the skin and connective tissues will be found to be colored very deep blue, the cartilages feebly colored, and the muscles green. The pancreas, the salivary glands, the fat, and the nervous system remain uncolored after moderate sized injections; but if the infusion be larger the gray substance of the brain is colored a dirty violet. A highly interesting feature, and one accidentally

observed in Professor Ehrlich's laboratory, is the complete filling of the system of bile capillaries in the liver, which is easily demonstrable in frozen sections.

A noteworthy feature of this injection is that it involves only the capillaries of the biliary system, which are almost uniformly distended, and thus give rise to a strikingly fine histological picture. These easily prepared pictures are, I think, superior to any staining of the bile capillaries by methods now in vogue, and demonstrate without difficulty the intracellular terminations of these vessels. Dr. W. R. Williams has, at my suggestion, worked with this method in my laboratory on the livers of animals which have been poisoned with phosphorus, with iodide of potassium and with toluylendiamine (1:3:4), with a view of studying the biliary capillaries in these conditions of poisoning. In normal animals subjected to infusions of indochromogen the bile and connective-tissues are stained blue, notwithstanding the violet color of the introduced dye. This blue dye, as it occurs in the bile, can be shown to differ in its chemical character from the indochromogen. In the experiments upon animals poisoned with toluylendiamine and subsequently infused with the indochromogen solution, the blue dye failed to find its way into the bile and the bile capillaries in the liver were only partially filled. A fuller report on the behavior of the indochromogen under pathological conditions will be given elsewhere.

If we bring a solution of dimethylparaphenylendiaminethiosulphonic acid into relation with the naphthaquinone sodiummonosulphonate instead of the disulphonate, we obtain a dye which differs from indochromogen S in several respects, and affords an instructive example of the influence of chemical constitution upon the distribution of organic substances in the living organism.

This dye has a purple-violet color, is less soluble than the indochromogen, and is considerably more toxic. If infused into living rabbits this coloring matter gives rise to a wholly different picture from that obtained from the indochromogen. The connective-tissues are colored faintly violet; the fat and the gray substance of the brain are dyed respectively purple-red and deep

purple. The pancreas, which in the indochromogen experiments was uncolored, is here dyed purple. This behavior in the organism can only be referred to the influence of the elimination of that second sulphonic acid radical, which, in the case of the indochromogen, is located in the second naphthaline nucleus. The acid character of the indochromogen was destroyed through the loss of this second sulphonic acid group, and the dye acquired certain characters that pertain to basic dyestuffs. Among these is the property to enter readily into the fat tissue and the gray substance of the central nervous system. This property is one which, as Professor Ehrlich first indicated, is apt to pertain to those basic dyes which readily diffuse into ether.

Although no thorough study of the toxicology of the naphthaquinone sulphonates has yet been made, it is certain that these bodies do not belong in the category of extremely poisonous substances. Thus a dose of 1 gram of the monosulphonate by mouth, while usually terminating fatally in a rabbit weighing 1500 grams, does not invariably produce death. A dose of this size is always followed by some prostration, diarrhœa, and frequent micturition. The urine contains both reduced and unreduced naphthaquinone sulphonate and sometimes hæmoglobin. The infusion of 0.1 gram intravenously in the course of thirty minutes is generally followed by death.

Dr. Park has very kindly investigated the bactericidal activity of the monosulphonate. The results are indicated in the following statement:

BACTERICIDAL STRENGTH OF NAPHTHAQUINONE MONOSULPHONATE IN WATERY SOLUTION UPON TYPHOID BACILLI FROM A CULTURE WHICH HAD BEEN KEPT ON ARTIFICIAL MEDIA FOR ONE YEAR.

Typhoid bacilli from bouillon culture, 50,000 to each cc. of distilled water or distilled water with disinfectant added; $\frac{1}{100}$ cc. amount plated.

Typhoid Bacilli present originally in all approximately 50,000.	After 5 min.	10 min.	30 min.	60 min.	20 hrs.
Distilled Water.....	50,000	50,000	50,000	50,000	35,000
" " and .1 % N.S.	50,000	50,000	50,000	40,000	■
" " and .5 % "	30,000	10,000	500	0	0
" " and 1. % "	0	0	0	0	0
" " and 1.5 % "	0	0	0	0	0

Colon Bacilli.	After 5 min.	10 min.	30 min.
Control in .00001 cc.....	52		
In .1 % N.S.....	38	37	45
In .5 % "	18	3	0
In 1 % "	0	0	0

B. pyocyaneus.	After 5 min.	10 min.
Control in .00001 cc.....	66	60
In .5 % N.S.....	0	0
In 1.0 % "	0	0

The bactericidal strength of the substance seemed about the same upon the typhoid and colon bacilli, the results in the .1 per cent seeming a little in favor of the colon bacillus being less sensitive. The larger figures at 30 minutes were undoubtedly due simply to the irregular distribution of the colon bacilli in the fluid, and did not imply a growth. The bacillus pyocyaneus was very much more sensitive, being killed by a .1-per-cent solution in five minutes.

In addition to the various biological uses of the napthaquinone compound which have already been enumerated, there are still others, the value of which cannot now be estimated owing to inadequate experience. For example, after the use of antipyretics, such as phenacetin and acetanilid, the urine contains a substance, probably para-amidophenol, which can be detected by means of its reaction with naphthaquinone sodium-monosulphonate. The study of the reactions of the urine with this substance under pathological conditions, and after the use of drugs and poisons, will doubtless in time become the subject of careful investigation, and it is possible that some medical applications will emerge from such a study.

Although it is probably no exaggeration to say that the naphthaquinone compound, which has formed the subject of discussion in these pages, is one of the most reactive color-producing substances at present known, it will not do to overlook the fact that its very lability must in itself have the drawback of depriving many of the reactions of any characteristic and specific features. In some cases we find that a whole group of compounds, like the primary aromatic amines, react in a similar manner, so that we have to deal with a class reaction for the amido group, rather than with specific reactions for individual substances. It also remains to be seen whether the usefulness of the reactions which

have been described will not suffer some restriction in those cases where we are dealing with a mixture of substances in solution, rather than solutions of chemically pure compounds. But notwithstanding these limitations, which are as yet not accurately definable, one is justified in predicting for the sulphonic acid derivatives of naphthaquinone a sphere of usefulness for the physiological chemist, as well as for the student of organic chemistry.

I wish to acknowledge the valuable help of Miss Louise M. Foster in aiding me in testing the substances mentioned in this paper, as well as very many others. To my friend, Prof. Paul Ehrlich, I feel deeply indebted for my introduction to the naphthaquinone sulphonates.

ON THE PREPARATION OF SALT-FREE CULTURE MEDIA AND THE GROWTH OF BACTERIA UPON THEM.

By ALONZO ENGLEBERT TAYLOR.

(*From the Hearst Laboratory of Pathology, University of California.*)

Some time ago I entered upon a study of the relations of different salts and nutrients to the metabolism of bacteria. The first step in the investigation lay in the preparation of a salt-free culture medium. A perusal of the literature indicated that the preparation of such a medium had never been really attempted with natural nutrients; and all the work upon the influence of particular salts upon bacteria is rendered of doubtful value by reason of the fact that the salt in the culture medium was not controlled or removed. For this failure technical reasons have been directly responsible. A culture medium containing natural nutrient materials cannot be controlled in its content of salt. Culture media composed of some amido body, as asparagin, plus a sugar, may be prepared in such a manner as to permit of a control of the salts. But by chemical procedures it is not possible to determine the saline content of a culture medium containing natural nitrogenous substances, nor is it possible to eliminate the salts. Thus far few results of importance have been secured in this investigation beyond the technical preparation of a salt-free medium; and these are herewith reported in the hope that they may prove of service to others engaged in research along similar lines.

PREPARATION OF SALT-FREE CULTURE MEDIA.

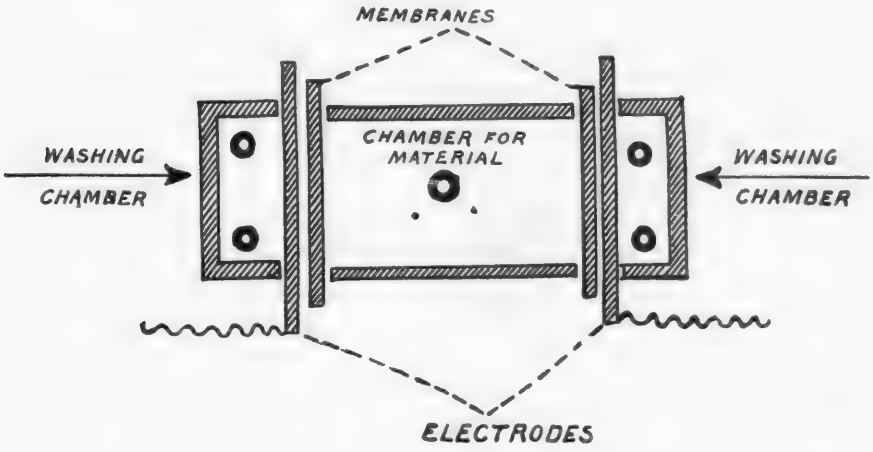
In the beginning a systematic attempt was made to prepare proteins free of salts. Casein may be prepared with an ash content as low as one-tenth of one per cent. Beyond this no chemical method will go. Such a casein is practically insoluble, and

even in the presence of calcium salts it exhibits such alterations in behavior as to suggest denaturation. Insoluble globulin, so-called, may be prepared with an ash content of about the same amount. This globulin, however, while quite soluble in alkaline or acid solution, becomes on manipulation and conservation so denaturated as to be entirely insoluble. Egg-albumin cannot be prepared so poor in salt. Fibrin may be freed of salt to about one-fourth of one per cent. The behavior of the proteoses is no better than that of the coagulable proteins. In fact, proteose is less tractable to purification by dialysis than is simple protein. Peptone may be prepared quite poor in salt by repeated precipitation with alcohol and finally with tannic acid. Dialysis is of little value, as the peptone itself diffuses to some extent, and some of the salts cling to the peptone with the greatest tenacity. The best available method consists in the isolation of the peptone by precipitation with iron and its final separation by alcohol according to the method of Siegfried. This author states that he has secured salt-free peptone by this method; but working with large quantities I have been unable to do so, as colloidal iron, and other salts as well, have always clung to the peptone. In any event this is of little consequence, since these substances (concerning the chemical individuality of which doubts are justified) are poor nutrients for bacteria.

Salt-free protein of the simple type of the protamines may be prepared without great difficulty. I have thus prepared the protamines from the salmon and the striped sea-bass, and also the histone from the star-fish. These all may be prepared so salt-free that the ash is not weighable. But they are not good nutrients for bacteria, and this statement is true for their protones. Moulds grow well on these protamines; bacteria grow poorly.

A salt-free medium was finally prepared according to an electro-chemical method suggested by Dr. F. G. Cottrell, Instructor in Physical Chemistry in this University. The method, which combines diffusion with electrolysis, had been previously employed for somewhat similar purposes, and has been used in particular by Dr. Cottrell for the preparation of pure colloids for

physico-chemical studies. The principle consists in the transportation of a powerful galvanic current through the material, whereby the cathions and anions wander respectively to their appropriate electrodes, and, passing through diffusion membranes to reach the electrodes, are removed by lavage of the electrodes with distilled water. Upon the efficiency of this lavage depends the success of the manipulation. The apparatus employed consists of a central glass cell of varying capacity (up to a half-litre), open at both ends and having a perforation through the wall for



the introduction of the material to be electrolysed. The open ends are covered with diffusion membrane of tested quality, back of which are placed sheets of fine platinum mesh, and on the outside of these are clamped tightly the outer glass cells that have openings to permit of the inflow and outflow of the washing fluid. All connections must be water-tight. The lavage chambers behind the platinum electrodes should be shallow, so as to ensure a frequent changing of the water. The connections with the current are made directly to the platinum mesh. The accompanying illustration will render further description superfluous.

When first set in operation the reaction may be rather violent and considerable heat may be evolved. If this be deemed undesirable the current (110 volt circuit) may be reduced by the insertion of resistance, as by the insertion of a few lamps, or the entire apparatus may be immersed in cold water. The greater portions of the salts are soon removed, together with acid or alkali, and as it is these ions that carry the current through the medium, the conductivity decreases as the removal of the salts is accomplished. Finally, the conductivity attains a constant minimum, which will be found to be somewhat above the conductivity of the distilled water employed in the preparation of the medium. This greater conductivity of the medium as contrasted with the distilled water is due to an electrical endosmosis, the particles of colloid being able to transport a small amount of the current. An amperemeter should be inserted into the circuit so that the progress of the reaction may be observed. It not only shows the close of the reaction in favorable instances, but also indicates the occurrence of disintegration of the substrate by the current, since under these circumstances the curve of conductivity in time will not exhibit a progressive descent, but will display wide fluctuations and variations. After every period of prolonged rest, as overnight, the conductivity is for a short time increased, only to drop to the previous level after a few moments. The more nearly complete the process, the less marked is this increase in conductivity after a period of rest. Obviously the method can be applied only to typical non-diffusible colloids.

In this way I have prepared a ten-per-cent gelatine medium. The gelatine was first crudely purified by soaking in weak alkali, and after it had become swollen it was repeatedly washed by decantation. It was then brought into solution by heat and precipitated by the addition of the least effective quantity of alcohol; in this way, while one loses gelatine, one retains less of salt than with precipitation with an excess of alcohol. This process was repeated once, and the final precipitate washed with alcohol and ether and dried, following which it could be weighed for the preparation of the ten-per-cent solution. The

gelatine was dissolved in water that had been twice distilled in a quartz apparatus under the utmost precautions: the conductivity at 16° was 1.1×10^{-6}. The final solution was submitted to electrolysis for five days, by which time the conductivity had reached a constant minimum. The conductivity was determined by the method of Kohlrausch, with the use of a standardized set of resistances. It was 8×10^{-6}—about, therefore, the conductivity of good double-distilled water, from which carbon dioxide has not been excluded during the distillation, and is about x 200 the conductivity of the purest water of Kohlrausch. During the preparation of the gelatine solution and its electrolysis, no attempt was made to exclude entirely carbon dioxide, and the final conductivity of the gelatine may be reasonably attributed in large part to this acid. The material was certainly not salt-free in the absolute sense, but it was very poor in salt.

Such a gelatine contains about two-tenths of one per cent of sulphur. This sulphur is in organic combination; it is a constituent of the gelatine molecule and cannot be detected unless the organic matter is destroyed. The material also contains a trace of phosphorus, likewise in organic combination. If some of the gelatine be digested with hydrochloric acid and potassium chlorate (free from phosphorus), the reactions for phosphoric acid are always to be demonstrated in the digestion fluid. Now the best analyses of gelatine (those of Faust, Paal, and Moerner) have not disclosed the presence of such phosphorus in the molecule of gelatine. It is in all probability best to be explained by the assumption that a trace of other organic matter has accompanied the gelatine, possibly nuclein or nucleo-proteid. In any event, the traces of phosphorus are not to be excluded from the best commercial gelatine. On incinerating a large quantity of the gelatine, after fusing with lye, the presence of the phosphoric and sulphuric acids may be demonstrated in the residue; but no other anions, chlorine in particular, are to be detected. Flame analysis fails to yield any signs of the presence of the ordinary alkali metals. The ash of ten grams of such gelatine, water-free, is not weighable.

Impure gelatine does not give the same result on electrolysis,

and decomposition always occurs. Apparently a certain degree of purity is necessary in order that the reaction shall not disintegrate the gelatine. If the method be employed on a good gelatine at a low temperature, the gelatine will lose nothing of its power of gelatification. At higher temperatures, however, the faculty of gelatification is rapidly lost, more rapidly, indeed, than would seem to be accounted for by the action of the heat alone. A finished gelatine product is absolutely neutral when tested with the Nernst gas chain, using hydrogen electrodes.

I am advised by Dr. Cottrell that, for reasons not yet clear, the method fails with agar-agar. The galactan does not become salt-free, but does lose its power of gelatification. The galactan is either dependent upon some mineral combination for its power of gelatification, or else it suffers during the electrolysis some disintegration attended with the loss of this attribute.

GROWTH OF BACTERIA ON SALT-FREE GELATINE.

This simple salt-free gelatine I have tested upon many bacteria. None of the ordinary saphrophytic or pathogenic bacteria will grow upon the medium. One large bacillus is able to develop upon this gelatine, though very slowly. This bacillus, which in some respects resembles the group of the hay bacillus, is a saphrophyte native to our city water, and since the spores are very resistant to heat it tends to contaminate all media sterilized by fractional sterilization. It forms on ordinary media yellow projecting colonies, is stained by Gram's method, ferments glucose, liquefies gelatine very slowly, produces traces of indol, does not coagulate milk, produces no acid reaction, and is feebly motile. Since the colonies project upward, it is possible to remove the top of a colony, and after suspending it in distilled water the germs may be plated upon the gelatine; from this plating a second suspension in distilled water is prepared, and in this way the danger of carrying over salts is avoided. The bacillus grows on the salt-free gelatine very slowly and months are required to produce a growth, which on ordinary gelatine would be developed in a week. After prolonged cultivation the micro-organism seems to become better inured to

the nutriment, and a more rapid growth is to be noted. Liquefaction, however, is much delayed. When the gelatine has become dried out (this desiccation is much slower than with the same gelatine before electrolysis) the culture dies.

The crucial experiments were carried out in quartz. The same results were secured with well-steamed Jena glass. Control tests with Jena glass showed that, after a proper steaming of the test tubes, water would not in a month dissolve enough of the glass at the temperature employed (20°) to increase measurably the conductivity of the water.

The growth of this bacillus is greatly retarded in an atmosphere free of carbon dioxide or of ammonia. From this it must be inferred that these gases are utilized in the metabolism of the plant. The retardation is most marked in an atmosphere free of carbon dioxide. Apart from the ammonia of the atmosphere the plant has only the nitrogen of the gelatine available for the necessities of its nitrogenous metabolism. Nitrogen is needed for the synthesis of common protein, the purin nucleus, and the pyrimidin ring,—the necessary constituents of the bacterial protoplasm. That ammonia may be utilized to these ends is of considerable interest, and though we are not in a position to speculate, it may be provisionally assumed that the process of building up from ammonia is in general the converse of that by which in higher organisms protein and nuclein are broken down. To what extent the molecule of gelatine must be disintegrated before it can furnish material suitable for these syntheses, can only be conjectured.

Beyond the carbon of the molecule of gelatine the medium contained no available carbon. The utilization of the atmospheric carbon dioxide classes the bacillus with higher plants. I am not acquainted with studies tending to show to what extent bacteria may utilize carbon dioxide for nutritional purposes. We do not know in what form the carbon exists in the molecule of gelatine and cannot even speculate upon what may be the steps in the process of its utilization.

For the synthesis of nuclein, phosphorus is needed. As stated the gelatine contained traces of phosphorus in organic com-

bination, sufficient doubtless for the needs of the nucleinic metabolism. The sulphur needed in the synthesis of the protein of the bacterial protoplasm was also available in the molecule of gelatine.

For practical purposes it may be stated that the atmospheric ammonia, and that derivable from the desintegration of the gelatine, were the only cathions present. To what extent the atmospheric ammonia figured as a cathion, and to what extent it was simply a nutriment cannot be determined. Substances that readily yield ammonia, such as amido bodies, are great promoters of growth of this micro-organism in the gelatine, from which the importance of ammonia as a nutrient substance may be deduced. That ammonia was the sole cathion present cannot be maintained in any absolute sense; hence the experiments ought simply to be interpreted as an illustration of the minimal cathion requirement for bacterial life. In an entirely similar manner the media were not in the absolute sense free of salt, and likewise the experiments should be accepted as an illustration of the minimal saline requirements for bacterial development.

Other peculiarities observed were the darker color of the pigment produced on these media and the absence of indol formation. Old cultures although faintly alkaline are much less alkaline than cultures grown upon ordinary media.